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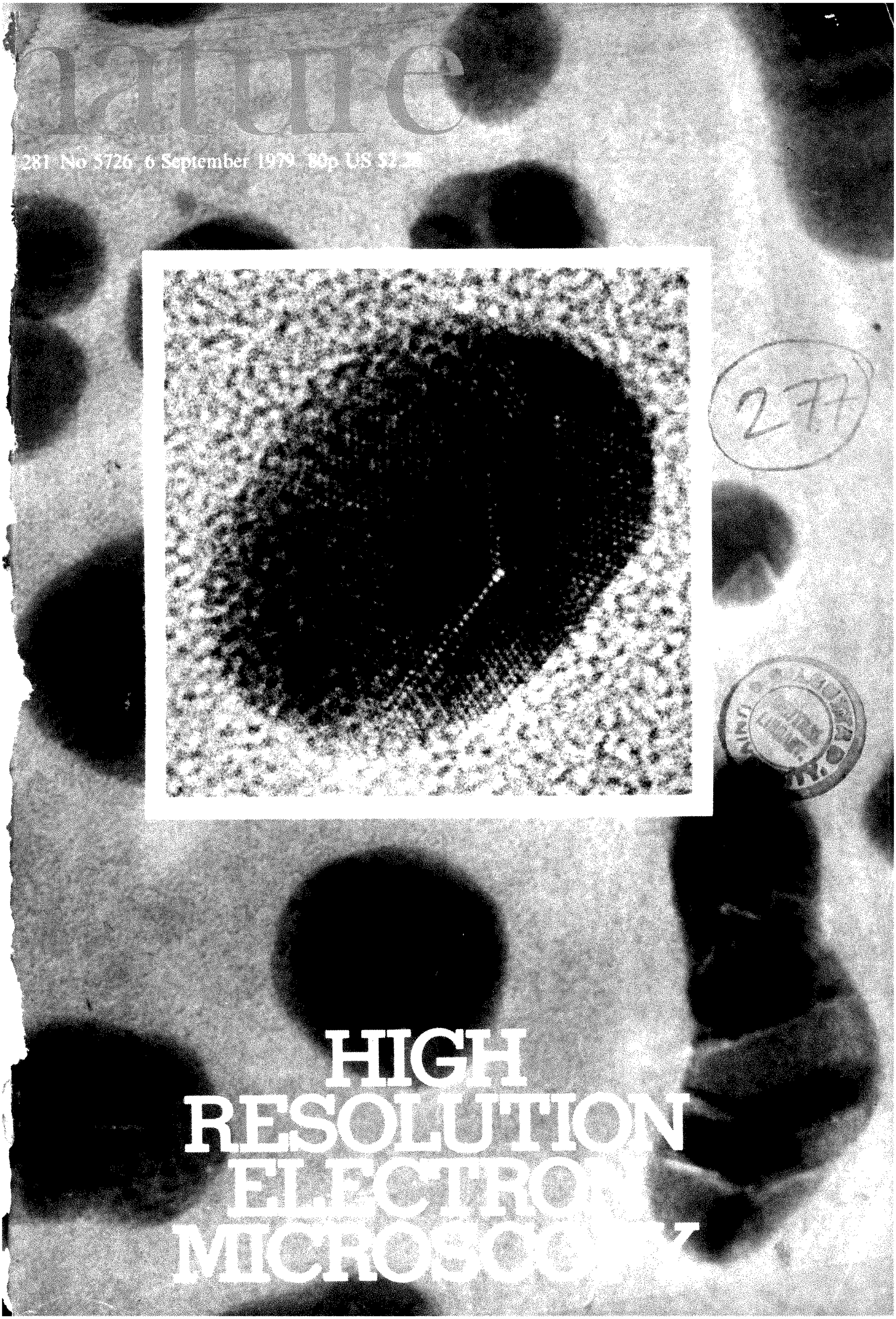

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Editorial Staff
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Publishing Director: Jenny Hughes

New York
175 Fifth Avenue, New York, NY 10010
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Robert Ubell (American Publisher)
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Telephone: (202) 737 2355 Telex: 64280
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6 September 1979

UNCSTD was a modest success

Developing countries have been told to take science and technology much more seriously — and have been given a little help to do so. Much depends on how they use the first $250 million.

AS the couple of thousand delegates who have attended the UN Conference on Science and Technology for Development (UNCSTD) in Vienna disperse, most will be asking themselves whether the conference was a success or a failure. For some, who came with very specific objectives such as to do business with other delegates, the answer will be equally specific. But for the vast majority who came with rather more diffuse expectations the answer will be complex. In the end, of course, no real assessment can be made until we can apply a historian's eye to it and ask whether the whole range of activities associated with UNCSTD — the national discussions, the regional conferences, the involvement of non-governmental organisations (NGOs), the pre-conference negotiations quite apart from UNCSTD itself — has significantly quickened the pace of development. And for that the historian must be sure that what 'development' means is how people themselves want to share in the intellectual and physical resources of the world. This means freedom not just for developing countries to design their own development strategies, free from the dictates of the developed world and its technology-salesmen. It also means freedom for the people of developing countries to choose not necessarily to go along with the development strategy worked out for them by a Westernised elite in their capital city. It means freedom for individuals to go their own separate ways in pursuit of individual development. So the influence of UNCSTD is hardly to be measured in a short space of time by adding together a few numbers.

In tangible terms, however, UNCSTD will leave behind a mountain of national reports, regional summaries, discussion papers — and a handful of documents debated at the conference itself. We report on subsequent pages on how these documents (the Programme of Action and a paper on science, technology, development and the future) were negotiated over the two weeks. Since the UN rule is to proceed by consensus if at all possible, they are necessarily broad generalisations and in many cases contain exhortations that individual countries will simply be unable to act upon. Negotiation is carried out by blocs and the interests of individual nations sometimes have to be surrendered for reasons of solidarity. Most publicity at the conference centred on the difficulty that the European Economic Community had in keeping to a unified position, particularly on questions of new financial support. But there is no reason to suppose that the Group of 77 (the developing nations), for which Tunisia acted, was any more united beneath the surface. The Group comprises nations as different as India, Brazil, Venezuela, The Gambia and Nepal, and so the diversity of need is enormous.

What they agreed upon

In the Programme of Action, delegates agreed, in essence, on the following:

- that the debate on the terms under which technology is transferred from the developed to developing world was not going to be resolved at UNCSTD; there are wide differences over questions of confidentiality, restrictive practices, the relevance of existing international agreements (at a time when the Group of 77 is promoting the New International Economic Order) and so on;
- that developing countries be exhorted amongst other things to:
  - formulate national science and technology policies;
  - make these policies as wide-ranging as possible;
  - establish policy-making bodies;
  - set up information networks, standardisation agencies, specialised institutions and so on;
  - formulate a technology transfer policy;
  - promote scientific associations, extension services, media reporting of science;
  - develop manpower policies and study the brain drain;
  - coordinate all this, and much besides, on a regional and international level;
- developed countries should help the developing countries along by supporting a new, additional funding facility under the aegis of the United Nations Development Programme. They should be invited to make pledges totalling $250 million over the two years to 1981. This facility would finance a broad range of activities aimed at strengthening the endogenous scientific and technological capacities of developing countries. The Group of 77 had initially asked for $2 billion per year by 1985;
- that a new UN inter-governmental committee oversee the implementation of the Programme of Action, and
- that a global information system be established.

In short, developing countries have been told to take science and technology much more seriously at a governmental level and to establish all sorts of new institutions. But the funding for it will very largely have to come from their own pockets. $250 million is not a large sum when divided amongst all potential applicants. And furthermore there is a strong feeling both in developed countries and in the poorer and smaller developing countries that when it comes to sharing out the money, the larger and relatively richer countries of the Third World will dominate the handouts, because they are more easily able to put up an impressive case, already having the right beginnings.

Role of scientists

The Programme of Action was almost entirely debated at the diplomatic level. How then did scientists and technologists fare at the conference? There had been an ambiguity about their role in UNCSTD right from the word go. Naturally it was not to be just another scientific conference. But how deeply were scientists to be drawn into participation in Vienna? It has been no secret that Joao Frank da Costa, the Secretary-General of the conference has had little enthusiasm for their involvement, and certainly issues such as transfer of technology, and institutional and funding arrangements within the United Nations system were ones on which relatively few scientists might have an informed view. On the other hand, scientists might have extremely clear views on global information networks, means of strengthening facilities in the developing world, research priorities and so on. In the event, however, the ambiguity persisted right to the end of the conference, and left many scientists disillusioned.

One reason for disappointment could have been the variability in the delegations. Some were manned almost entirely by civil servants, others carried a professor or two and a very few (such as West Germany, Austria, France and the United States) made any serious effort to be multi-dimensional by incorporating politicians, academics, industrialists and trades-unionists. There was thus a feeling amongst scientists that they did not constitute a critical mass capable of making their presence felt. This sentiment undoubtedly coloured the discussions in the working group on science, technology, development and the future. Under certain circumstances it would have been possible for this working group's report to have provided the perfect technical counterbalance to the administrative and structural Programme of Action — in effect presenting a pair of papers outlining possibilities and how they might be achieved. In practice this was not to be.

Some of the responsibility for this must undoubtedly be placed on ACAST, the UN's Advisory Committee on Science and Technology. The committee hastily put together a colloquium of 300 scientists in Vienna for the week before UNCSTD. Creditable as such an occasion may have been (and there were a majority of developing-world scientists at it), it undoubtedly siphoned much of the scientific interest away from UNCSTD. And since the working papers for it were prepared by the UN's special agencies, the colloquium already had the veneer of a UN activity before it started. Its final report merely confirmed this, being the sort of document that would be very acceptable in UN circles but which lacked a sense of novelty or character.

The UNCSTD working group started off by falling into the trap of wanting to submit a similarly dull document. But eventually a small group of scientists, clearly horrified at what they were in danger of having to put their name to, forced at least a modest revision (after the years of preparation and millions of dollars poured into UNCSTD, the group's activities were heavily curtailed by lack of time!). Ultimately the report did give a more satisfactory indication of how science and technology could contribute — with particular reference to the need to involve the young and to make positive efforts to encourage women. The role of the United States delegation was crucial — even during late-night negotiating sessions when almost every other delegation was either absent or fielding one representative, the US team would be up to ten in number.

Modest momentum

The cause of development is ultimately in the hands not of delegates to conferences but of politicians apportioning resources and of those at the cutting edge. Has enough come from UNCSTD to give them new direction, support and drive? The Chinese at UNCSTD produced a very apt proverb "don't add yet more flowers to the bouquet; send more charcoal in snowy weather". Will the conference be remembered just for verbal bouquets, or are developed countries prepared to send more tangible help? The level of financial support over the next two years leaves the question open, although the establishment of the funding facility itself must be regarded as a modest step forward in international collaboration. But much depends on just how effectively developing countries put together their proposals in the coming months. It would be easy to spend the money on large capital-intensive projects — grandiose new universities and research laboratories lavishly equipped with equipment but short on running expenses and staff, particularly at the technician level. By 1981 such demands could already have given a bloated and inappropriate image to the whole project. It is better that developing countries (and particularly the poorer developing countries) take the opportunity slowly and thoughtfully to lay down the foundations of a real science and technology policy. Much of the money would be better spent on encouraging communication and discussion than in bricks and mortar.

The outcome of UNCSTD has never been seriously in doubt — the Group of 77 have had to sacrifice their rosier dreams, developed countries have had to make some relatively minor concessions. What matters now is that the modest amount of momentum acquired is not squandered in the years ahead.

A slightly bolder approach

As long as *Nature* has existed, letters reporting important scientific advances have formed the backbone of the journal. The length of these communications has grown steadily with the years as scientists have felt under greater pressure to give sufficient detail to allow a critical evaluation, but their style has remained remarkably unchanged.

We are often asked why we do not fall into line with almost every other journal and publish an abstract with each letter — and our answer has been that we have tried to preserve in the letters section the sort of directness that people achieve when writing to each other. But we go on to add that there is nothing to prevent anyone writing a first paragraph that encapsulates the whole story — background, summary of the work done, consequences; something more valuable than an abstract because it gives context.

For the past few years we have gradually been trying to persuade authors to improve their first paragraphs, if necessary with some help from our sub-editors. Now we take the experiment a bit further. From this week's issue, the first paragraph of each letter will be printed in a bolder face. We hope this may provide some little help to those very many of our readers who turn to *Nature* to keep in touch with a wider range of science than their own specialisation. We hope too that our authors will be encouraged to put even more thought into getting the first paragraph right. And the writer of this leader might practise what he preaches. □

UNCSTD w agreed

isions were agreed at the session of the United Nations on Science and Technology ment which ended in the al hours of last Saturday morning for was to set up a new inter tates of committee on science a mend the development, open al system for the UN. The seco development establishmen of this are being science a an interim fund based and, ributions, with an initial $250 million for the two 980–81. Both these decisions ications of the Group of 77's demands.

e Group of 77, for example, had ginally proposed that the intergovernmental committee be set up by, and report back to, the General Assembly of the UN. But the US had insisted that the committee be established by the UN's Economic and Social Council (ECOSOC).

A compromise was suggested by the Common Market countries and eventually accepted by the conference, along lines drawn up by the British delegation. The new committee will be set up by the General Assembly, but it will report back to the General Assembly through ECOSOC. And the latter, while not permitted to alter the reports, will be able to comment.

The new committee will play an important role within the UN system. How effective it will be will depend on factors such as the strength and status of the support it is given from the UN, which is yet to be decided. One of its main purposes, according to the wording of the final conference decision, will be to "help the General Assembly formulate guidelines for harmonising the policies of the United Nations system in regard to scientific and technological activities".

Specific responsibilities will include: identifying priorities for activities within the programme of action agreed by the conference (now referred to as the Vienna programme of action), preparing a plan for carrying out the programme, and monitoring activities and programmes related to science and technology within the UN system.

A particularly important function of the committee will be to "give directives and policy-making guidance" to the new financing system which the conference proposed that the UN should establish (an earlier proposal from the Group of 77 had been that the committee should "direct" the way that the *financing* system was operated, *but this* language was rejected as too strong).

The financing system agreed is considerably less than the original demand of the Group of 77. This was for a target of two billion dollars by 1985 and four billion dollars by 1990 reached through "automatic" contributions mainly from developed countries.

The main opposition from the developed countries to this proposal was based more on the manner in which contributions were to be made than on the size of the fund. Almost all objected to the idea of "automatic" contributions based on factors such as the balance of trade in manufactured goods — a type of international tax which was strongly resisted — and it was agreed that at least initially all contributions will be voluntary. Other countries, in particular various members of the EEC, were initially opposed to the idea of a fund at all but were eventually convinced to accept the general consensus.

The purpose of the proposed global financial system, according to the conference resolution, will be to "finance a broad range of activities aimed at strengthening the endogenous scientific and technological capacities of developing countries and, in particular, assist in implementing the programme of action".

It was agreed by all conference participants that factors to be taken into account in determining the nature and level of the system's resources should include: the asymmetry of technological capacity between developed and developing countries, the need for predictability and continuous flow of financial resources, the need for substantial resources additional to those existing within the UN system and finally the need for untied external resources.

Since it will take some time to make the arrangements for this financing system, an interim fund, to be administered by the United Nations Development Programme, will be created: and a target for voluntary contributions to this fund has been set at "no less than $250 million" over the two years 1980 and 1981.

A conference will be held before the end of the year at which countries will indicate how much they are prepared to contribute to the interim fund. The main potential donors are the US and Saudi Arabia, with promises from France and a number of smaller European countries. Precisely how the interim fund will be spent remains to be worked out. But it is thought that most of it will go towards helping to build up scientific infrastructures in developing countries.

David Dickson

What scientists did in Vienna

WHILST there was no doubt that the main focus of attention at UNCSTD would be the debate over the Programme of Action, with its associated battles over institutions, money and so on, it was planned that there would be a parallel session to use the assembled scientific and technological expertise to good effect. This session had two fields to look at: new science and technology for overcoming obstacles to development; and science and technology for the future. The working group was chaired by M. Malitza of Romania.

The group had at its disposal national papers, together with two specially commissioned studies and the reports of several conferences. It also had freshly to hand a report from ACAST, the UN's Advisory Committee on the Application of Science and Technology which had met in Vienna the previous week.

The working group (which comprised as many delegates as cared to show up) always seemed to be loaded with disadvantages. It did not start meeting till the fourth day of the conference, and then in a building not within walking distance of the main conference centre; it was presented with a bland ACAST report which seemed to convince the working group that its report should be equally platitudinous; and whilst a few delegations (France, Germany, Austria, United States in particular) had a good complement of scientists and technologists, many could field at most one, more often none at all.

The first draft of the report reflected

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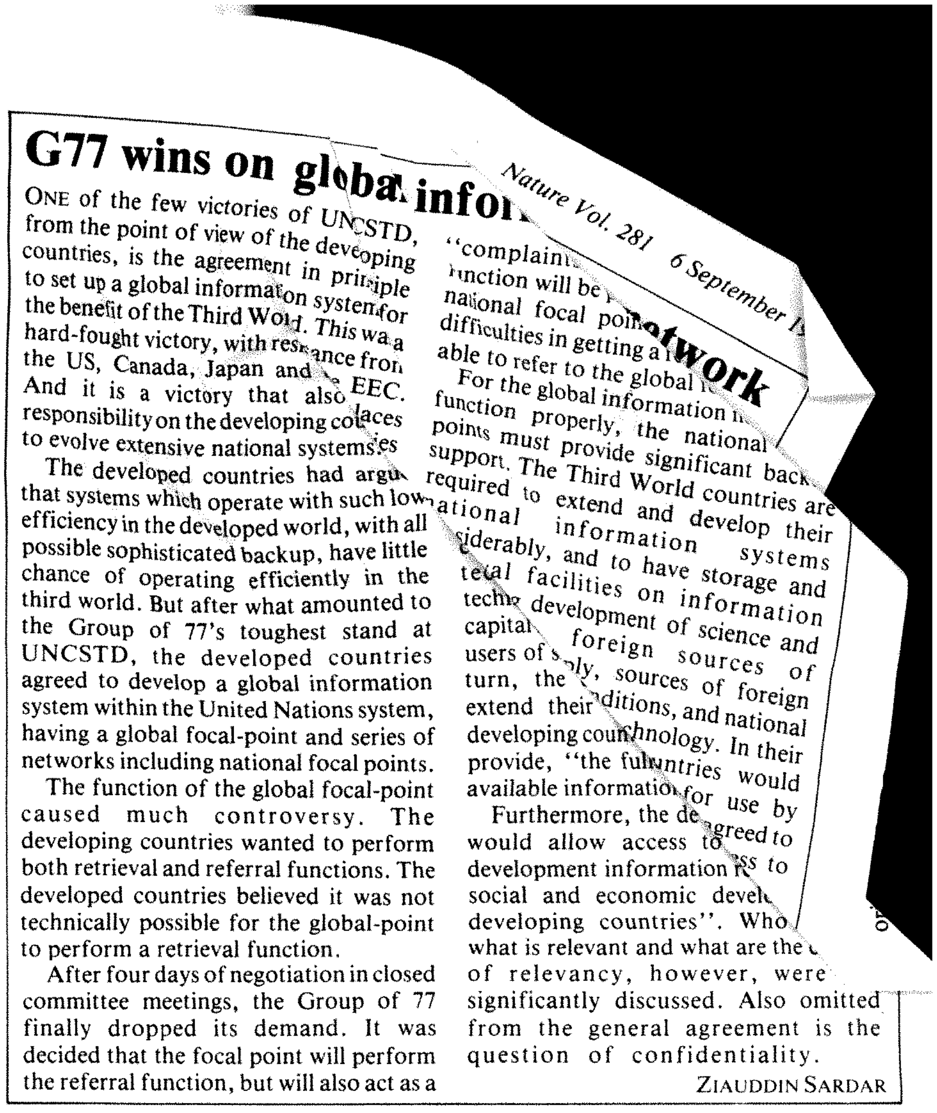

G77 wins on gl·ba· infor· ·twork

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ONE of the few victories of UNCSTD,
from the point of view of the deveoping
countries, is the agreement in prin·iple
to set up a global informaton systen for
the benefit of the Third Wo1d. This wa a
hard-fought victory, with res ance fron
the US, Canada, Japan and EEC.
And it is a victory that also laces
responsibility on the developing co ies
to evolve extensive national systems
The developed countries had argu
that systems which operate with such low
efficiency in the developed world, with all
possible sophisticated backup, have little
chance of operating efficiently in the
third world. But after what amounted to
the Group of 77's toughest stand at
UNCSTD, the developed countries
agreed to develop a global information
system within the United Nations system,
having a global focal-point and series of
networks including national focal points.
The function of the global focal-point
caused much controversy. The
developing countries wanted to perform
both retrieval and referral functions. The
developed countries believed it was not
technically possible for the global-point
to perform a retrieval function.
After four days of negotiation in closed
committee meetings, the Group of 77
finally dropped its demand. It was
decided that the focal point will perform
the referral function, but will also act as a

"complain
unction will be
national focal poi
difficulties in getting a
able to refer to the global
For the global information
function properly, the national
points must provide significant back
support. The Third World countries are
required to extend and develop their
ational information systems
siderably, and to have storage and
tel facilities on information
techn development of science and
capital foreign sources of
users of s ly, sources of foreign
turn, the ditions, and national
extend their hnology. In their
developing coun ntries would
provide, "the ful for use by
available informatio agreed to
Furthermore, the de ss to
would allow access to
development information
social and economic devel
developing countries". Who
what is relevant and what are the
of relevancy, however, were
significantly discussed. Also omitted
from the general agreement is the
question of confidentiality.

ZIAUDDIN SARDAR

these weaknesses. The United States, which had not been one of the 6 members of the drafting group and which claimed it was having difficulty in getting its views considered, was particularly severe in its strictures that the report lacked 'soul'. As one of its delegates put it, "we have put a big effort into this conference; how can we defend this document to our scientists and technologists back home?" The British, too, were voicing concern both publicly and privately that "with all the expertise here in Vienna" nothing more helpful was emerging for consideration in planning future development programmes.

Certainly much of the document merely repeated the same general principles as were being negotiated more formally in the Programme of Action elsewhere. But by this stage there was not enough time left to start again from scratch. Nor would many developing country representatives have been pleased at the prospect, as they were more in need of a general statement of principles to put to the government back home rather than a 'soulful' document.

In the event, and amidst much lobbying by the US, a second draft was produced which left the Americans moderately satisfied without causing problems amongst developing countries. Some of the key points are outlined below.

Science: 'Science policy should be mainly linked to long term objectives'. 'Science and technology . . . should be consistent with national aspirations and objectives'. 'Science of the future must include in its concerns the uplifting of the welfare of the total human being' (the first version had contained the phrase 'basic science should be left free to follow its own internal dynamics').

Obstacles: 'One of the most urgent questions is disarmament . . . more effect should be given to analysing in what ways ongoing research and technical application now directed towards war could be adjusted for peaceful applications'. 'The brain drain of the most skilful scientists and technologists from developing countries creates a serious obstacle to their own scientific and technological development . . . developed countries should assist by actively discouraging a brain drain from developing countries'. This last phrase was concocted as an alternative to the more loaded phrase 'should assist by not enticing scientists and technologists from developing countries', to which some developed countries took exception. But at least one delegate was wondering privately whether 'discouraging' the brain drain did not contravene agreements on the free-flow of scientists.

The United States would additionally have liked a reference to the population problem, but could not get a consensus.

Objectives: 'All countries should develop endogenous capabilities to assess the trends of science and the consequences of technological options'. 'Developing countries have to carry out their own studies of global problems so as to bring their own perspective to bear on the world *problematique.*' 'Goals include overcoming poverty and seeking ways to reduce the disparities that exist between developed and developing societies in, for example, life expectancy, infant mortality and education'. A stronger version speaking of 'halving by the year 2000 the disparities' could not attract a consensus.

Resources: 'As concerns R and D activities in developing countries, every effort should be made by the world community to raise the level of these activities from the present very low level to a substantially increased level. It was the considered view of many delegations that a more quantitative goal should be set and they felt that this should be of the order of 20% to 25% of global world research and development expenditure by the year 2000'. Countries such as India and Bangladesh were keen to have a target, but there were doubts amongst some countries about the reasonableness of these targets or even the precise definition of the terms used.

Action: The building up of endogenous capabilities requires 'massive expansion of the educational system in order to provide access to primary, secondary, technical and higher education to most, if not all, the populations, male and female equally'.

Governments in developing countries should provide support both for existing and new centres of excellence 'dedicated to both basic research and applied research of relevance to their societies'.

Scientists in developed countries 'should be encouraged and helped by their governments to take a more active interest in solving problems in developing countries . . . exchange of scientists and technologists between developed and developing countries should be encouraged . . . informal communications between scientists based on mutual interest are also among the powerful instruments which can be used to help in problem solving'.

Specifics: The working group made sure, in a footnote, that just about every branch of science and technology with the remotest relevance to development received a mention. But it also identified in the main text some rapidly developing fields such as information technology, non-conventional generation of energy (including fusion), biotechnologies and marine sciences.

Women and young scientists: 'Technological development often affects men and women differently and the intro-

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duction of new technologies has tended to have an adverse impact on the latter, thereby lessening their earnings and social status. It is therefore of the utmost interest to society that in future the full participation of women be ensured in the planning and setting of priorities for research and development as well as in activities relating to the design, choice and application of science and technology for development. They should also be provided with equal access to scientific and technological training and professional career opportunities'.

'Rapid development of science and technology throughout the world will depend in part on the younger men and women who can be brought into these fields, involved in decision making bodies and given full opportunity to use their intelligence and skills'.

Institutions: 'There is a need to restructure and strengthen the international scientific and technological system and the role of international intergovernmental organisations in such a way as to permit such organisations to act as effective instruments in satisfying the priority objectives of member states'.

'An appropriate UN body on science and technology, and other UN agencies competent in this matter' should make recommendations regarding changes in the structure of science and technology to 'service the needs and problems of the peoples of the world, with special regard to the needs of developing countries'. Originally this recommendation spoke only of the new intergovernmental committee that was being established elsewhere in the conference, but the Soviet delegate, in a rare intervention, asked for the involvement of other UN agencies. This possibly keeps the door open for UNESCO and ACAST, both of which had been widely thought to have lost considerable influence as a result of this conference.

David Davies

EEC countries attempt to speak with one voice

ONE of the most significant consequences of the UNCSTD conference is that, for almost the first time, the nine member countries of the European Economic Community were required to speak with a single voice on the appropriate international policies for applying science and technology to development.

At times, EEC maneouvring to achieve this unity resembled those of a cox-less, novice rowing-eight (Luxembourg played a relatively minor role in the negotiations), with everyone heading roughly in the same direction, but with substantially different ideas about commitment and timing. Furthermore, the political requirement for solidarity during formal negotiations with the developing countries meant that many harsh words were said to colleagues behind closed committee doors.

The outcome, however, was at least a partial victory for political cohesion. And this will inevitably have important consequences for the future role played by EEC countries in encouraging particular forms of economic and political development in the Third World.

The pressure to speak with a common voice added a third political factor to the two which have traditionally dominated national discussions of foreign aid policy: on the one hand, the domestic implications, both political and economic, of spending public money on foreign aid, on the other, the international gains to be made by spending this money in a particular way.

A fourth factor played a less explicit, but no less significant, role during the conference negotiations. This was the knowledge that the US had committed itself to ensuring the approval of an interim $250 million fund, with particular executive responsibilities given to the United Nations Development Programme, and investigating the setting up of a long-term international funding system for science and technology for development.

Caught between these four different sources of political pressure, each member country of the EEC was forced to weigh up their relative importance in its own particular circumstances — and decide its negotiating strategy accordingly. At the one extreme Denmark had, at the beginning of the conference, surprised some of its EEC colleagues by advocating immediate commitment to the US-backed package. Compromise was "imperative" to make UNCSTD successful, the Danish Minister for Foreign Affairs, Mrs Lise Ostergaard, told the plenary session.

Similar indications of support came from the Netherlands and Italy. Holland was eager for domestic political reasons not to appear to be backing away from its reputation for generosity in foreign aid, despite economic difficulties.

In contrast, the three major economic partners in the EEC — Britain, France and Germany — each had different reservations about the US-backed package. Britain's position was made clear by the Minister for Overseas Development, Mr Neil Marten, who told the conference that recent drastic cuts in public spending meant that Britain had no money to contribute to any new fund, and that in any case Britain had "serious doubts" about the value of any new financial mechanism or the separation of funds for science and technology for development.

At the same time, however, the British delegation was keen to maintain the political unity of the EEC's negotiating positions and not to be seen as the only country which was sticking out against a consensus.

Germany's position was more complicated. Politically it agreed with the US's view about the need for "cooperation" rather than "confrontation" in economic relations with the developing countries, and about how science and technology agreements can help achieve this goal. It also accepted the need for EEC unity, and could not complain about lack of funds.

For domestic political reasons, however, the German government was reluctant to provide funds to a programme whose precise activities had not been spelt out in detail. It tried — unsuccessfully — to get the EEC to back the idea of contributing directly to a World Bank research programme on renewable energy resources for developing countries.

Finally France, the maverick in the group, was happy to be seen increasing its foreign aid contributions, but less happy about a sectoral fund inside the UNDP. The French annoyed some other EEC delegation members by announcing to them two days before the end of the conference its opposition to the new fund.

Despite such divergences of opinion, by the end of the conference a consensus had been reached to support the US/UNDP package in principle (see page 2). At the closing session, however, it was publicly acknowledged that there remained differences between the EEC members. Speaking on behalf of the whole group as current president of the EEC, the head of the Irish delegation said that six countries — Belgium, West Germany, France, Ireland, Luxembourg and Britain — wished to make it clear that they maintained "their well-known reservation on the efficacity of any sectoral fund for science and technology for development". However, in the interests of achieving a consensus, the statement said, these six countries had not opposed the arrangements for an interim fund, once it had been established that the contributions would be on a voluntary basis.

No-one within the EEC delegation was entirely happy with the outcome. But it was generally accepted that, given the pressures under which the group was working the outcome as a political exercise was fair.

Whether the result will be of overall benefit to the developing countries is another question. Some EEC delegates point out that by agreeing to accept a consensus, some countries adopted a more conciliatory position than they might otherwise have done. Developing country critics, however, also argued that through such consensus-building, countries which might otherwise wish to adopt a more radical stance in their general approach to north-south relationships might be persuaded against doing so.

David Dickson

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Very high radiation levels found in Swedish houses

FOLLOWING the discovery of extremely high concentrations of radioactive radon daughter elements in Swedish houses, a government committee has proposed measures to allow maximum concentrations far higher than would be internationally acceptable. The radon producing the daughters derives not only from radium in the soil and rock on which the houses are built, but also from radium in building materials.

The immediate cause of the high levels recently recorded, however, is energy-saving. Enthusiasm for energy conservation has led many people to tape over their windows to keep the warmth inside; consequently ventilation rates have dived. With a ventilation rate of one air exchange every two hours, even the worst radon daughter levels are greatly reduced; but some of the houses measured have had only 0.2 air exchanges every two hours.

To overcome the conflict between energy saving and health, the committee suggests the installation of air exchangers in which the outgoing air would warm up the incoming air. Discussions are currently being held to decide how much financial assistance could be paid to house-holders for the necessary alterations, but at the moment it looks as though the amount will be small.

From the radon point of view, the worst building material used in Sweden is aerated concrete made from alum shale. Its manufacture was banned in 1974 for this reason, but it had been a popular building material for 45 years. At the other extreme is wood which contains practically no radium. Ironically enough, the houses whose high radioactivity led to the committee of enquiry were built of wood — but constructed on slag heaps left after the mining of alum shale. Only a small proportion of risk houses have so far been identified, and campaigns are being organised to find others. Current estimates of the numbers of houses that will have to be altered run as high as 15,000.

The International Committee on Radiation Protection recommends that no member of the general public should be exposed for many years to an annual dose equivalent of more than 1 millisievert (mSv) of radiation, excluding natural background and medical sources. This level is estimated to give a lung cancer rate of one person in every thousand exposed for 60 years. There is as yet no internationally-agreed level for exposure to radon in houses. In the average Swedish house, the concentration of radon daughters gives a dose equivalent of 3 mSv/yr, assuming a person spends 80% of time indoors — and the worst cases found in the present investigation are as high as 100 mSv/yr. "If nothing is done, our present building situation may cause up to 1100 cases of cancer in about 30 years", says the Director of the Radiation Protection Institute, Professor Bo Lindell.

The committee proposes that nobody in existing buildings should, over the next five years, be exposed to a radon daughter level of more than 2000 bequerel (Bq) year/m^3, a total dose equivalent of 285 mSv over 5 years. Existing houses should be altered before their inhabitants have received this total dose. This exposure will, it is estimated, mean that three people in every hundred will develop lung cancer after 60 years. As far as new buildings go, the committee proposes radon daughter concentrations of 70 Bq/m^3 (9 mSv/yr) and at this exposure level, one person in every hundred will develop lung cancer after 60 years. According to the committee, these dose rates are the best that can be achieved at the moment, given the level of radioactivity in Swedish rock, soil and building materials.

The number of people who will develop cancer because of the high radon daughter levels may however be less than these estimates. According to a spokesman at the Radiation Protection Institute, current research into the risks of contracting cancer at certain concentrations of radon daughters shows that the risks to the ordinary man may have been over-estimated. The risk estimates are based on studies of uranium miners, the concentrations around miners who developed cancer being extrapolated to the ordinary population. But these studies did not take into account the fact there is always more particulate matter in the air in mines than in ordinary houses. As radon daughters enter the body attached to airbone dust, the risk to the general public may have been overestimated.

Wendy Barnaby

Women in science: some improvement, but still a long way to go

There has been a considerable improvement in the status of women PhDs in academic science over the past decade, but further substantial gains are still needed before equal opportinuty is fully realised, according to a report published recently by a committee of the US National Academy of Sciences.

On the positive side, the report, prepared by the academy's committee on the education and employment of women in science and engineering, says that in the past decade women's share of all science doctorates has doubled, from 9% to 18%, and is still increasing in all fields of science.

Furthermore women are occupying a growing proportion of research faculty positions, particularly in the larger institutions; in the top 25 research universities, for example, women account for 35% of the growth of science faculties since 1973.

However there remain several areas of concern. One is that there continues to be a disproportionate number of women in two kinds of position: part-time instructors or lecturers which are outside the tenure stream offer little chance for productive research; and postdoctoral or research staff positions.

There are also areas in which the discrepancy between salaries for male and female research workers is giving continued cause for concern. The report points out, for example, that although the difference in postdoctoral stipends decreased from 5.7% to 4.9% between 1973 and 1975, it had increased again to 7.5% by 1977.

"On the basis of available data, it appears likely that at least a large part of the salary differences between men and women postdoctorals derives from bias. At this level no significant differences in overall ability or promise can be documented, and male and female scientists should be rewarded equally for comparable work. Systematic salary differences at this early career stage are important not only for their immediate relevance to equity, but also as a portent of future status," says the report.

Among the major conclusions of the report are that:

- rank for rank, women faculty continued to be tenured less often than men. For all ranks, 72% of the men but only 46% of the women hold tenure appointments, and this disparity is increasing
- in relation to the pools of new PhDs in the various fields, chemistry and mathematics employ far lower proportions of women faculty than other fields
- women account for all of the net growth in science faculty at the assistant professor rank for the top 50 US universities (by R & D expenditures) and for nearly half of the increase in all other institutions
- wide field of variations in rank, salary, and tenure distribution for women faculty compared to men suggest that an assumed lack of mobility of married women is at most contributory rather than a primary reason for women's evident disadvantage.

The continued plight of women in science was emphasised at recent hearings before Senator Edward Kennedy's health subcommittee by Ms Anne Briscoe of the Association for Women in Science, who claimed that despite the results of affirmative action programmes, sexist attitudes were still deep-rooted. The health subcommittee is currently discussing a "Women in Science and Technology Equal Opportunity Act". ☐

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news in brief

Silicon production costs 'reduced 90%': A new process, developed by a group of researchers at the Stanford Research Institute, California, may reduce the cost of silicon by as much as 90%, an SRI publication has claimed (*The Leading Edge,* summer 1979, p22). Thus, by 1986, 1 kg of silicon could cost as little as $5, says the article. At the moment, 1kg of silicon costs $60. Costs could be further offset by the sale of sodium flouride, a by-product of the new process, for other industrial uses. The new manufacturing method is a one-step process producing relatively pure silicon, with sodium flouride as a by-product. The necessary chemical reactions take place in one vessel and the cheapness and ease of the new process are further enhanced by the fact that the reaction is exothermic and therefore requires no outside fuel source. The main beneficiary of a new breakthrough in the manufacture of silicon would be solar energy using cheap silicon cells. However, the new process is yet to be tested on an industrial scale.

Development need not endanger the environment: Environmentally 'safe' development can be carried out by all nations according to Dr Mostafa Tolba, (right) Executive Director of the United Nations Environment Programme (UNEP). In an interview with *Siren*, UNEP's Regional Seas Programme newspaper, Dr Tolba said "appropriate environmental management should be a common practice to both poor and rich nations, and should be the meeting ground of common objectives." Developing countries should seek a solution to their environmental problems in the process of development itself. "It is not so much a question of financial resources necessary to meet environmental problems, as it is the undertaking of rational decisions based on a long-term integrated concept of development."

Dr Tolba felt that the regional approach to environmental problems was one of the most successful. UNEP's Regional Seas Programme is "one of the best of our programmes if not just the best". This programme aims to protect the enclosed seas and coastal environment where some of the highest levels of pollution are found. Compatible research will be carried out throughout these regional seas enabling a communal solution to the problem to be made. This, together with a common resolve among coastal states towards problems affecting them all, are the two most important practical achievements of UNEP's Oceans Programme according to Dr Tolba.

Aldermaston deaths: Two former workers at the UK Atomic Weapons Research Establishment, Aldermaston died from cancer last month with more than the permitted level of plutonium in their lungs. Internal organs from both mens' bodies have been sent to Windscale for tests, but it is not yet certain whether radiation caused their deaths. Kenneth Cummins, 49, had been at AWRE for 14 years and Albert Newman, 51, had worked there for 26 years. Both worked in the "active areas" of the establishment and were among the 70 men claiming damages against the Ministry of Defence for plutonium contamination (19 July, p184). Parts of Aldermaston were closed last year after 12 workers were found to have more than the permitted level of radiation in their bodies. Before these areas can be re-opened, new ventilation methods must be installed and more safety and maintenance workers must be employed in accordance with the findings of the Pochin report which examined radiological health and safety at the establishment and was published at the end of last year.

Finnish reactor delayed over safety requirements: Delays in commissioning the second set of the Loviisa nuclear power station are, it appears, due to the higher safety requirements demanded by Finland. The equipment for Loviisa, produced in the Soviet Union, includes a number of modifications not considered necessary for Soviet home consumption. These include a containment vessel and a shield to prevent internal corrosion of the reactor's pressure chamber.

In mid-June, the Finnish power authority Imatran Voima announced that the loading of the second set had been postponed, "since talks between Finnish and Soviet specialists on the removal of the sheild had not yet been concluded." It was not clear at that stage why an anti-corrosion shield should need to be removed at all, although the Finnish media stressed that Imatram Voima "wished to observe strict safety standards" and "in case the chamber needs to be repaired" were ready to postpone the loading till August "or ever later".

The most recent news from Imatran Voima reveals that the authority had applied to charge the set in June, but the Radiation Protection Institute had delayed permission, after cracks had been found in the "inner anti-corrosion coating of the vessel" (presumably the "sheild" referred to in earlier statements). These cracks, it was alleged, were due to slag "inclusions". The Soviet suppliers have offered an explanation of these faults.

Air pollution complaints increase: The number of complaints about air pollution made against industrial works in the UK increased in 1977 despite an overall reduction in air pollution, according to a Health and Safety Executive report published recently. Mineral works were the worst offenders accounting for 97 of the complaints. The Chief Inspector of HM Alkali and Clean Air Inspectorate, Frank Ireland, said "We receive far more complaints today about far less pollution compared with conditions even 10 years ago when the situation was much worse." More inspections, stricter standards and greater public awareness probably accounted for this increase.

There was also a slower rate of implementation than expected of new emission standards set by the Alkali and Clean Air Inspectorate in 1977 due to the economic depression. Some old plants were kept active to keep staff in employment, particularly in the iron and steel industries, and other industries were allowed longer to implement the new standards so that they could spend scarce resources on creating or maintaining existing jobs. Existing standards, however, were never relaxed and in many cases emissions were considerably better than requirements. *Health and Safety: Industrial Air Pollution* 1977, HMSO (London) £3.

Pioneer 11 discovers new ring of Saturn: The Pioneer 11 spacecraft, which successfully flew past Saturn last Saturday, has discovered a fifth ring, the "F" ring, lying outside those already known and circling the planet. Attempts to find evidence of a broader, more diffuse "E" ring have given only ambiguous results so far. The gap between the "A" and "F" rings has now been labelled the Pioneer Gap. Photographs returned from the space craft also indicate the presence of a dark blue jet stream with greenish tinges near Saturn's north polar region and of a possible new satellite. The satellite which appears faintly in photographs has yet to be positively identified: it may be a newly discovered moon or possibly some known satellite appearing unexpectedly.

Short wave radio fools Fire Brigade: A short wave radio in the Israeli Embassy in London is now thought to be the cause of the radiation detected recently at Kensington Fire Station (30 August, page 715). The National Radiological Protection Board called in by the London Fire Brigade found no unusual levels of radiation and thought interference from radio waves may have caused the fire brigade equipment to register radioactivity.

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Students meet before the conference: social values are not being taught with technology

Scientists and clergy debate global problems

Scientists and theologians from the First and Third Worlds met recently to discuss the social impact of western science and technology. Much of the debate was heated as **Dr A R Peacocke**, of Clare College, Cambridge University, reports.

AN unprecedented world conference on 'Faith, science and the future', organised by the World Council of Churches, was held at the Massachusetts Institute of Technology last July. It drew many threads of the WCC's previous work together and was structured around four central themes: science and faith, questions of ethics, of political economy, and plans for Christian action in relation to science and technology.

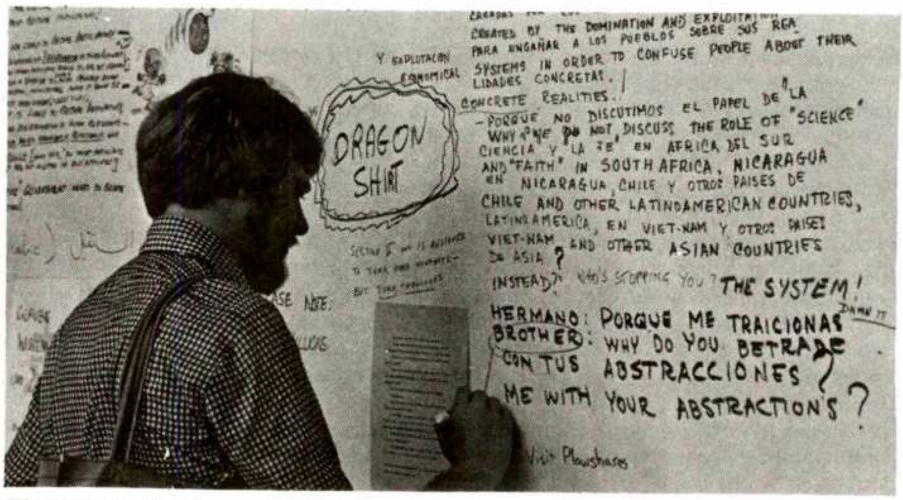
Three hundred and thirteen delegates from 56 countries attended. About half of them were scientists and technologists and half were theologians, pastors, social philosophers and politicians. More than 100 students also attended. Many church affiliations were represented and a few representatives from other religions, including Islam and Buddhism, were present. With such a mix, it is not surprised that strong feelings were expressed, especially when topics for discussion included nuclear weapons, genetic engineering, nuclear and other forms of energy and the impact of western science and technology.

Delegates from the 'Third World' were about a third of the total and rapidly constituted themselves as a pressure group. It soon became clear that they experienced 'science' primarily as technology, a technology moreover that is having an enormous and disruptive effect on their societies, with the transnational corporations cast as the principle agents of their ills. Their frustration and sense of exploitation was expressed in a resolution (not a resolution of the whole conference) in which a number of them said "we denounce the historical and current use of science and technology by industrially and technically advanced societies, to serve military and economic interests which have brought about great sufferings to the people of the Third World. This has been done in the guise of an ideology of objectivity and value-free pursuit of truth". These attitudes produced first shock and then strong opposition from western (or, more accurately, northern) scientists and others present who regarded the pursuit of knowledge of the natural world as valuable in itself and knowledge, once achieved, as ethically neutral.

This particular tension became apparent early in the conference in the contrast between the account of science, given by Professor R Hanbury Brown (astronomer from Sydney University), as "a social activity in which we seek to discover and understand the natural world" and that of Dr Rubem Alves (philosopher, Brazil) for whom "most of the explanations that science proposes about itself are not only untrue; they are dangerous", as dangerous as a wolf to Third World lambs. There was clearly a wide gulf of misunderstanding between these two typical western and Third World perspectives on science — and indeed technology should have been substituted for science in many of the discussions and even in the conference title.

It is hard to judge if this gulf was really bridged, but the western-trained scientists, technologists, economists and theologians were uneasy with the inadequate treatment, especially in the section meetings, of conceptual and critical issues such as the status of scientific models, the limitations of scientific knowledge and how scientific research projects are engendered and financed. However this was inevitable in such a mixed gathering and the concern was expressed in section recommendations which requested the WCC to initiate smaller and more professional study conferences on particular assigned problems. These kinds of recommendation are more likely to be implemented than worthy, but doomed-to-be-ineffectual, instructions to those outside the churches such as the recommendations of one section to governments on nuclear energy, and those of another to "governments, industrial companies, universities and scientists" to make scientific and technological information more accessible to all citizens.

Matching in intensity the Third World issue, was the desire of the delegates to


DRAGON SHIT
CREATED BY THE DOMINATION AND EXPLOITATION SOBRE SUS REA
PARA ENGAÑAR A LOS PUEBLOS
CONFUSE PEOPLE ABOUT THEIR
SYSTEMS IN ORDER TO
LIDADES CONCRETAS.
CONCRETE REALITIES.
-PORQUE NO DISCUTIMOS EL PAPEL DE LA
WHY DO WE NOT DISCUSS THE ROLE OF "SCIENCE"
CIENCIA Y LA FE EN AFRICA DEL SUR
AND "FAITH" IN SOUTH AFRICA, NICARAGUA
EN NICARAGUA, CHILE Y OTROS PAISES DE
CHILE AND OTHER LATINOAMERICAN COUNTRIES,
LATINOAMERICA, EN VIET-NAM Y OTROS PAISES
VIET-NAM AND OTHER ASIAN COUNTRIES
DE ASIA ?
INSTEAD?
WHO'S STOPPING YOU?
THE SYSTEM!
HERMANO: PORQUE ME TRAICIONAS
DAMN IT
BROTHER: WHY DO YOU BETRAY
CON TUS ABSTRACCIONES ?
ME WITH YOUR ABSTRACTIONS ?
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The conference 'wallnewspaper'

show their unambiguous support for nuclear disarmament. Confessing "the part played by science in the development of weapons of mass destruction and the failure of the churches to oppose it", the delegates were unanimous in their endorsement of a resolution (drafted by a committee chaired by Dr J Habgood, Bishop of Durham) that called for full implementation of SALT II, advocated SALT III, supported UN initiatives on disarmament and opposed the development of new nuclear weapons. However, one proposal for unilateral disarmament was overwhelmingly defeated.

The other aspect of the nuclear problem, the 'peaceful' uses of atomic energy, also led to lively controversy. Professor David Rose, of MIT, argued for the continued use of nuclear energy at least until solar energy is developed, which means in practice, for several decades. But Professor J Rossel, a Swiss physicist, argued equally strongly against its use. Not surprisingly the section report concerned with this issue was divided and at the plenary session a keen debate resulted in the passing of a resolution, by a 129-45 vote, that called for a 5-year moratorium on the construction of nuclear power plants. My impression was that this majority included a large number of votes from among the 100 student representatives who, although neither strictly delegates of their churches, nor appointed as experts, had full voting rights.

The students, from 55 nations, had met before the conference at Wellesley College, Boston, to discuss the key issues. They asserted *inter alia* that science education is not tying social values to the teaching of technology and this theme was also heard when they reported their views to the main meeting. They were again especially critical of science education which they saw as an elitist weapon used to preserve power and institutionalised values.

This raises the wider question of how representative the conference was. But, representative of whom? Certainly it voiced the profound concerns about science and technology of many active members of the Christian churches across the world, which, it must be stressed, are increasing rapidly in the Third World. It is difficult for such a conference to claim to be 'representative', but it might nevertheless aspire to leadership of opinion resulting in action. If it does, the hopes over many years of the devoted staff of the WCC (and one thinks particularly of Dr P Abrecht, the director of the Church and Society unit that was responsible) will have been justified. And, in the end, the most remarkable thing about this unique conference is not so much its plethora of resolutions and its section reports (yet to be published) but the fact that it was held at all — and in MIT. The relation of the Christian churches to modern science and technology can never be quite the same again. ☐

Can biogas provide energy for India's rural poor —

or will it benefit only richest 10-12% of rural families? **Anil Agarwal** reports on the Janata government's plans to promote the use of biogas plants

INDIA has the world's second largest bio-gas programme, surpassed only by China, and its interest in bio-gas has probably the longest history. Although the first bio-gas plants were designed in 1939, the Khadi and Village Industries Commission was able to establish only 6,000 plants by 1974. The energy crisis of that year, which sent the price of kerosene soaring above the purchasing power of rural Indians and pushed them further into the environmentally disastrous use of firewood, persuaded the government of India to promote bio-gas plants more seriously.

In the last five years, over 75,000 bio-gas plants have been set up, and during the sixth five year plan (1978-83), the government hopes to establish 500,000 plants at a total cost of Rs2175 million (Rs17.7 = £1). About a third of this sum will be provided by the KVIC and the Ministry of Agriculture as grants, the rest being raised through loans from banks. These plants will create an annual production capacity equivalent to 750 million litres of kerosene. In addition, they will generate about 12 million tonnes of organic manure, which otherwise would have been burnt as cowdung cakes for fuel.

The programme should provide jobs for over 2,000 educated unemployed people and some 40,000 skilled and semi-skilled workers.

But compared to the total bio-gas potential within the country, the current rate of utilisation is extremely slow, much slower than China, which is reported to have set up over seven million bio-gas plants in a few years. Mr T.L. Sankar, secretary of Planning Commission's working group on energy policy, estimates that India has a potential of at least 18,750,000 family size bio-gas plants and 560,000 community size plants (with daily capacities of 1.7 and 142 cubic metres of gas respectively). While the family size plants could be used by richer rural households (with 4-5 head of cattle), the community size plant could supply energy to poorer households.

If this potential were realised by 1990, bio-gas could supply India with an energy equivalent to nearly 44% of its projected electricity consumption, and reduce its projected consumption of coal by 15% and firewood by 79%. The use of firewood would be reduced to an environmentally safe level, saving forestry expenditure, and the organic manure by-product would also reduce expenditure on chemical fertilisers. Although the investment required to adopt this option would be very high, about Rs 66,000 million. Mr Sankar believes that an energy policy that places high emphasis on bio-gas plants would have the "highest cost-benefit ratio".

Earthscan

Mound of cowdung cakes: a traditional source of fuel

To promote the use of bio-gas plants, the KVIC has started a self-employment scheme under which educated unemployed persons are being given training in installation and commissioning. They are paid Rs180 for supervising the installation of each gas plant. The KVIC takes a deposit of Rs50 as security before they begin work and retains 25% of their supervision charges until the total security reaches Rs500. These 'motivators' are expected to rectify all defects in the plants they have supervised, within the first three months of commissioning, at their own cost. Through this scheme, the KVIC hopes that greater care will be taken at the time of construction and thus maintenance and breakdown problems should be reduced; it claims that this scheme has given "very good results". Over 1500 motivators have already been trained.

Problems of managing community plants

But achieving Mr Sankar's target could be very difficult. There appear to be serious socio-economic obstacles to the widespread use of bio-gas plants. Past experience has shown that small family-size plants can be used only by the richest 10-12% of rural families. The large-scale use of bio-gas plants could drive poorer families to a greater reliance on the dwindling

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supplies of firewood, as their present cowdung fuel becomes unobtainable.

Ironically, while the Chinese have unhesitatingly promoted the use of small plants, appropriate technologists in India have argued that only large community plants can benefit the Indian rural poor. But such plants require great managerial talents for their maintenance and operation, for the organisation of the collection of cowdung, valuation of the dung which would have to be purchased, and sale of the gas and manure generated. Furthermore, sociologists still have to explain what motivated those farmers who have bought bio-gas plants. While some reports say that the initiative comes largely from women, who like bio-gas as a clean fuel, other reports claim that farmers buy bio-gas plants because it provides them with organic manure for their fields, and regard the gas only as a by-product.

A conventional KVIC bio-gas plant with a mild steel gas holder

Earthscan

With financial assistance from UNICEF, two large prototype plants ($30m^3$ and $45m^3$ respectively) have been built in the village of Fatehsingh ka Purwa in Etawah district. Bio-gas from these plants will provide cooking energy for the 200 inhabitants of the village as well as energy for running threshers, chaff cutters, mini-four mills, and for pumping water and generating electricity for street and home lighting. Fatehsingh ka Purwa is mainly a village of cowherds and the ratio of cattle to man is relatively high, which makes it possible for bio-gas alone to supply energy for so many activities. The total cost of the project is Rs158,000. Each family will receive slurry from the bio-gas plants in proportion to the cowdung it supplies. Bio-gas will be supplied free for two years and thereafter the villagers are expected to contribute. Few villagers at the moment appear to be prepared to pay anything for their energy, the concept of paying for fuel being traditionally alien to them. But they should eventually pay Rs10-15 every month per family. This is only one-tenth the depreciation and interest on the project, which thus involves a considerable subsidy. The Planning, Research and Action Division (PRAD) of the State of Uttar Pradesh, which is organising the project, has hired a manager for the plants and a trust of villagers has been formed to oversee it.

Meanwhile, the Department of Science and Technology is supporting an extensive R&D programme to develop cheaper designs of bio-gas plants and identify new feedstocks which can replace or supplement cowdung. Two important designs have been developed: one by PRAD which is an improved version of a Chinese design supplied to Pakistan, and the other an indigenous design developed by the KVIC.

In the standard KVIC design, the fermentation pit (also called the digester) and the gas holder are two separate structures, the mild steel gas holder floating over the fermentation pit. This design is efficient, gas-tight and provides gas at a constant pressure, but the gas holder is susceptible to corrosion and cannot be made in the village.

In the Chinese design the fermentation pit and gas holder are combined into one brick structure, which makes it cheaper and easier to construct and maintain a bio-gas plant with a village's own resources. Although the initial versions suffered badly from gas leakages, PRAD workers now claim that their improved design is not only as efficient as the KVIC design but also half its cost. About 110 plants have been built in Uttar Pradesh and Rajasthan, and PRAD is organising training courses for constructing these plants, now called Janata (People's) Bio-gas Plants.

KVIC is meanwhile seriously evaluating its own new design in which both the digester and the gas holder are made of ferrocement. The digester is made up of a series of modular ferrocement rings which fit into each other, and the ferrocement gas holder slides into the digester. The farmer, has nothing to do but dig a suitable size pit. Some 100 such experimental plants have been set up and they are reported to be operating very satisfactorily. The ferrocement plants are about 20% cheaper than the conventional plants, but still slightly dearer than that of the Janata plants.

A hybrid design, incorporating a ferrocement gas holder with a conventional brick and mortar digester has been developed by the Structural Engineering Research Centre at Roorkee, and several voluntary groups are also working on new bio-gas plant designs. Mr J.C. Kapur of Kapur Solar Farms in New Delhi has developed a combined system of solar water heaters and bio-gas plants. The output of bio-gas plants falls drastically during the winter months, when minimum temperatures in northern India drop to near zero, and these are the months when more cooking energy is required. Solar heated water can keep the temperature in the digester at an optimum level and thus maintain the gas output. The bio-gas plant provides energy for cooking, to generate electricity for lighting, and also to take over from the solar plant for air conditioning when solar intensity is low. Kapur foresees a totally automatic interactive system.

Searching for new feedstocks

Simultaneously, several efforts are being made in various agricultural universities and research centres to find new feedstocks for bio-gas plants. The KVIC itself is experimenting with plants that use agricultural wastes (straw from wheat or rice, for example) with not more than 5% dung. Another possibility is water hyacinth, a weed that grows prolifically in various parts of India. The PRAD workers have already found that a mixture of water hyacinth and dung in the ratio of 2:3, gives a gas output increased by 40-50% and also a slurry richer in nitrogen, phosphates and potash. The KVIC is also experimenting with bio-gas plants which use urine as a feedstock: in some states the villagers are beginning to show less aversion to using human wastes in bio-gas plants.

The KVIC proposes to set up five regional bio-gas experimental centres to try out various types of bio-gas plant with a range of feedstocks in different agro-climatic, socio-economic and resource conditions. This type of research should help to identify the best designs for different regions.

But despite this vast programme, bio-gas researchers continue to be treated as second-class citizens in energy research. Work at the Gobar Gas Research Station of PRAD in Ajitmal, for instance, is treated by research workers as an interim arrangement until they find a better job. Researchers still receive low salaries and suffer from job insecurity, conditions under which no Indian nuclear researcher, for instance, would ever work. Successive Prime Ministers have stated that bio-gas is a matter of national interest, and India still needs to implement fully this good intention. □

news and views

Earth's cratering rate

from David W. Hughes

THE hysteria over Skylab's return to Earth and the unease engendered by the fact that about 600 of the 4,500 or so orbiting pieces of space hardware re-enter the atmosphere every year pales into insignificance against the shock that would follow the impact of an asteroid and the subsequent cratering event. How often are these impact craters produced? Well, a body capable of forming a crater with a diameter over 1 km hits the Earth every 1,400 years. Craters of 10 km diameter and more can be produced every 140,000 years assuming the impacting object hits land and not sea. These figures come from two reviews of the subject by R.A.F. Grieve and P.B. Robertson and Grieve and M.R. Dence of the Canadian Department of Energy, Mines and Resources and published in a recent issue of *Icarus* (**38**, 212; 230; 1979).

Terrestrial impact craters are fairly common and Fig. 1 shows their world wide distribution. Twelve of the structures shown in Fig. 1 are classified as proven meteorite craters because meteorite fragments have been found in their vicinity. The remaining 75 are 'probable' meteorite craters recognised as such because the target rocks have suffered shock metamorphic effects. It is obvious from the uneven distribution that the probability of their preservation and detection varies considerably from place to place. The majority occur in North America and Europe, areas with relatively stable geology and also with an active population of crater searchers. Erosion and geological processes progressively erase craters. For example a 20 km structure is only recognisable as a probable impact crater for a maximum of 600 million years whereas a 10 km structure usually has a lifetime of only 300 million years.

Morphologically there are simple bowl-like craters with an uplifted and overturned rim and complex craters with uplifted central peaks and slumped or depressed rims. Normally the simple craters have diameters of less than 4 km, the larger ones being complex.

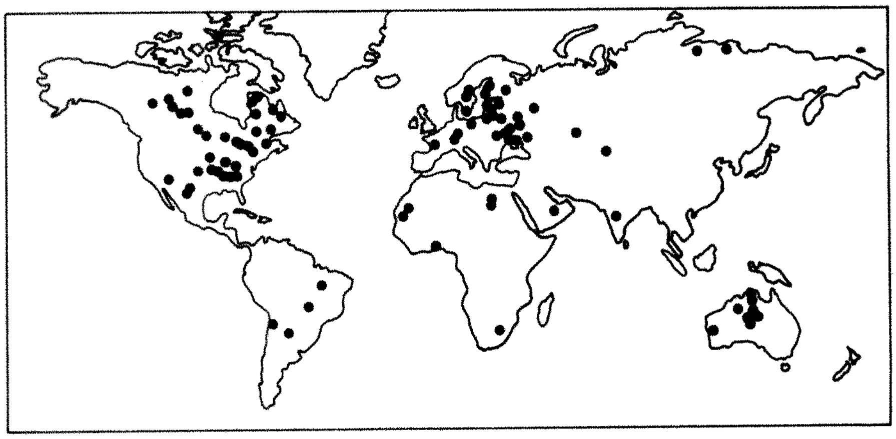

Fig. 1 The world wide distribution of impact craters (after Grieve and Robertson).

Plotting the number of craters, N, which have diameters D greater than D as a function of D it can be seen that $N \propto D^{-2}$ for D larger than 20 km. The curve departs from linearity below this size because small craters have a shorter lifetime and are less likely to be recognised. It is highly probable that the $N \propto D^{-2}$ relationship actually extends down to crater diameters of about 1 km. Martian craters obey an $N \propto D^{-2}$ law. Large lunar craters lie between $N \propto D^{-1.8}$ and $N \propto D^{-2}$. This law can also be justified by combining the mass distribution of the causative asteroids (the number of asteroids more massive than M is proportional to $M^{-0.6}$) with the relationship between the crater diameter and the kinetic energy, E, of the impacting body ($D \propto E^{0.29}$).

To calculate the cratering rate one needs ideally a large area of the Earth's landmass which has a single age of origin, preferably as old as possible, and which has undergone a minimal and uniform level of subsequent modifying geological activity. Two areas approximate to this. The first is the North American stable region that lies between the Rockies and the Appalachians and the Quachita and the Arctic. The area is 1.25×10^7 km^2 and the exposure age varies from 450 million years in the north to about 300 million years in the south. It contains 33 impact structures over 1 km in diameter, the estimated production rate being $(0.36 \pm 0.1) \times 10^{-14}$ km^{-2} yr^{-1} for craters with $D > 1$ km. The second area is the Northern European plain lying between the Scandinavian Caledonides, the Urals, the Carpathians and the Caucasus. Here there are 20 craters with $D > 1$ km. Over an area of 4.5×10^6 km^2. As with North America the exposure age varies from 450 million years in the north to 300 million years in the south resulting in a production rate of $(0.35 \pm 0.13) \times 10^{-14}$ km^{-2} yr^{-1} for craters with $D > 20$ km. Combining these two production rates gives the equation

$$\log_{10}\phi = -11.85 - 2\log_{10}D \qquad (1)$$

where ϕ is the production rate (km^{-2} yr^{-1}) of craters with diameters greater than D km. This equation is definitely valid down to diameters of 20 km and probably valid down to diameters of 1 km. The results can also be interpreted as indicating that this cratering rate has remained reasonably constant over the last 3.4×10^9 yr. Equation (1) shows that, assuming the Earth's surface was all land, a 1 km crater would be produced every 1400 yr and a 10 km crater every 140,000 yr.

We must be thankful that two-thirds of the globe is covered by water and, of the remaining land mass, the frozen wastes, jungles, deserts and mountains account for over 90% of the area. ☐

David W. Hughes is a Lecturer in the Department of Physics, University of Sheffield.

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Gene mapping at the crossroads

by Mary Lindley

THE human gene map is said to be poised for rapid expansion, during the next few years, as the techniques of molecular biology and immunology take firm root in this traditional domain of geneticists and cell biologists. At a recent reunion in Edinburgh*, 150 of the aficionados agreed that they have now identified the positions of about 350 genes on the human chromosomes. Although the current rate of increase is a mere two or three genes per month, the atmosphere was full of confidence that this will accelerate rapidly, making a complete human gene map a possibility within the lifetime of some of the younger members of the gene mapping fraternity.

One of the most promising strategies involves the combination of some of the familiar tools of cytogenetics with techniques developed to investigate the fine structure of both prokaryotic and eukaryotic genes. By such methods the ß-globin gene, already believed to be on chromosome 11, has been localised more precisely by D. Housman (Massachusetts Institute of Technology) and colleagues. They used cells from hybrid clones each of which contained the Chinese hamster genome together with a section of human chromosome 11. To identify the section carrying the ß-globin gene they extracted the DNA from cells of each clone, treated it with a restriction endonuclease and separated the resulting fragments on agarose gel. The separated fragments were transferred onto nitrocellulose filters and hybridised to a radioactively labelled cloned human ß-globin DNA probe. The pattern of hybridisation revealed by autoradiography showed that the gene for human ß-globin is carried on a section of the short arm of chromosome 11. Because the gene for ß-globin is known to be linked to those for γ, δ, and ε globins, all four genes must be carried on this chromosomal region.

By a similar procedure, B.R. Migeon and colleagues (Johns Hopkins University School of Medicine, Baltimore) have used hybrid cells containing the mouse genome and a human chromosome resulting from a translocation between chromosome 11 and the X chromosome (*Proc. natn. Acad. Sci. U.S.A.* in the press). They were able to select for the presence or absence of the human chromosome because it carried a functioning gene for the enzyme hypoxanthine guanine phosphoribosyl transferase, which the mouse cells lacked. These experiments have further confirmed that the ß and δ-globin genes are carried on human chromosome 11, and show that they are not on the segment involved in the translocation.

The application of 'restriction mapping' to increasing numbers of genes now depends on the rate at which cloned probes can be producd and characterised. F.H. Ruddle (Yale University) predicted that restriction mapping will become the predominant method for mapping genes, and that the major part of the human gene map could be known by the end of this century.

Speed, convenience and scope for fine resolution should give restriction mapping an advantage over *in situ* hybridisation, which has recently revealed further information about the arrangement of the histone genes on chromosome 7. Using labelled probes of complementary RNA, prepared from cloned sea urchin genes, D.M. Steffensen (University of Illinois, Urbana) and J.J. Yunis and M.E. Chandler (University of Minnesota Medical School) have both localised the five human histone genes to the long arm of this chromosome. Their results, however, do not indicate precisely the same positions, with the latter group placing the cluster of genes nearer to the end of the long arm (Chandler *et al. Science,* in the press). The difference may be due to the difficulty of interpreting the pattern of silver grains in the final, autoradiographic stage of this procedure.

Another promising line of enquiry for the future is provided by cell surface molecules which can be recognised by biochemical and immunological techniques and correlated with the presence of particular chromosomes. A. de la Chapelle (Folkhälser Institute of Genetics, Helsinki) and colleagues have followed up previous studies which indicated an association between monosomy for chromosome 7 and defective granulocyte chemotaxis (Rutuu *et al. Nature* **265**, 146; 1977). Treating the defective monosomic granuloctyes with a radioactive label specific for glycoproteins, the Helsinki group has shown that these blood cells have much less than the normal amount of one particular glycoprotein. This suggests that a gene on chromosome 7 codes for a glycoprotein associated with chemotaxis. The same chromosome may also be involved in the expression of receptors for epidermal growth factor, according to N. Shimizu and colleagues (University of Arizona, Tucson). They have correlated the presence of this chromosome in hybrid cells with the ability to bind epidermal growth factor.

Indirect immunofluorescence, used by C.K. Eun and H.P. Klinger (Albert Einstein College of Medicine, New York), has suggested that the expression of fibronectin on cell surfaces is affected by chromosome 11. This contrasts with the suggestion of Owerbach *et al.* (*Proc. natn. Acad. Sci. U.S.A.* **75**, 5640; 1978), who labelled surface proteins by lactoperoxidase-catalysed iodination and correlated the presence of fibronectin with chromosome 8. The disagreement arises again in the results of a similar study by M. Smith (Mount Sinai School of Medicine, New York) and colleagues who found that hybrid cells could be positive for fibronectin while lacking glutathione reductase which is associated with chromosome 8. The genetics of fibronectin it seems may be complex, and possibly may involve genes on various chromosomes. On the other hand, the discrepancy may be resolved by the application of more refined techniques.

Another promising area is represented by two reports of genes that influence the expression of oncornaviruses. Using human–mouse cell hybrids, C.E. Wright and T.B. Shows (Roswell Park Memorial Institute, Buffalo) have found a gene on human chromosome 11 that induces the expression of a mouse xenotropic oncornavirus and another on chromosome 15 involved in the induction of endogenous mouse N-tropic oncornavirus. They call these genes *BVIX* and *BVIN*, respectively, and have found that they are carried on human chromosomes homologous to mouse chromosome regions that also carry genes affecting the expression of oncornaviruses.

This suggestion of genetic conservation during evolution is just one of many examples that are emerging as gene maps are developed for different mammalian species. As well as those of mouse, rabbit and various primates, genes are now being assigned to the chromosomes of cow, sheep, pig, cat, dog and horse. As would be expected, these are providing further evidence of the conservation of the X chromosome during evolution. It is also becoming increasingly clear that other linkage groups have been strongly conserved, especially between the great apes and man. Yunis and Chandler, for example, have extended their *in situ* hybridisation study to chimpanzee, gorilla and orangutan and localised the histone genes on homologous regions of the counterparts of human chromosome 7.

Similarly J.J. Garver (State University, Leiden) and colleagues have localised the major histocompatibility complexes of chimpanzee and rhesus monkey on a chromosome homologous with human chromosome 6, which carries the HLA complex. P.A. Lalley and colleagues (Oak Ridge National Laboratory) have found that some genes on human chromosomes 1 and 6 are correspondingly linked in the baboon, indicating conservation. On the other hand, they interpret their study of the baboon counterpart of human chromosome 2, as a sign that extensive chromosomal rearrangements have occurred during the evolution of this chromosome. Many more such insights should come as the various gene maps develop. ☐

Mary Lindley is an Assistant Editor of Nature.

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*The fifth International Workshop on Human Gene Mapping was held in Edinburgh on 9-13 July 1979. It was organised by H.J. Evans, MRC Clinical and Population Cytogenetics Unit, Edinburgh. The abstracts and committee reports will be published in *Cytogenetics and Cell Genetics.*

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Hotspots and African mantle

from Peter J. Smith

EIGHT years ago Morgan (*Nature* **230**, 42; 1971) proposed the existence of hotspots, which he envisaged as manifestations at the Earth's surface of narrow plumes of hot mantle material rising from below. Ever since these supposed lithospheric penetrations have been a perpetual source of controversy. Do they exist at all? And if they do, how many are there — a few, or tens, or even hundreds? Are they really linked to deep mantle plumes? If so, are the plume positions laterally fixed in the mantle or do they (and hence the hotspots) move relative to each other? Do hotspots-plumes really account for the phenomena (Hawaiian volcanism, for example) often attributed to them? And so are linear volcanic age trends (the Hawaiian-Emperor chain, for example), hotspot traces, resulting from the motion of tectonic plates over (stationary?) plumes beneath?

Nothing like a consensus has yet emerged on any of these questions. Which makes it all the more fascinating to observe that Thiessen *et al.* (*Geology* **7**, 263; 1979) have had the temerity to superimpose this set of controversies on another uncertainty, namely, the mobility/immobility of Africa. A few years ago Minster *et al.* (*Geophys. J.* **36**, 541; 1974) concluded that for the past 10 million years Africa has been moving at the rate of about 2 cm a year. Burke and Wilson (*Nature*, **239**, 397; 1972), on the other hand, have taken the view that for the past 25 million years or so Africa has been stationary with respect to the underlying mantle; and there is much to be said for this suggestion. Palaeomagnetic data appear to rule out any major latitude changes during this period; and about 25 million years ago there was a dramatic increase in African volcanism, implying a continent that had come to rest relative to its underlying volcanic sources.

Of course, to anyone strongly committed to the existence of mantle plumes, African volcanism and African hotspots are synonymous; and to that extent Burke and his colleagues made the hotspot–immobility connection right from the start. According to this vision, the increase in African volcanism about 25 million years ago and the continued high level of volcanic activity since then simply reflect the relative ease with which mantle plumes may pierce stationary, as opposed to moving, lithosphere. Then regions of high relief/elevation without associated volcanisms are regarded as nothing less than incomplete hotspots, or highspots, resulting from the same underlying processes as the hotspots proper. Thus Africa's distinctive basin and swell topography, where the swells are seen as hotspots/highspots, becomes a natural consequence of the continent's immobility.

Those who remain unconvinced about the reality of mantle plumes, or who accept the existence of only a few of them, will find this proliferation of hotspots and quasi-hotspots outrageous. Nevertheless, it is interesting to see how Thiessen and his colleagues (who, incidentally, include Burke) combine hotspots and a stationary Africa into a consistent picture and then go on to develop the model in terms of its implication for the underlying mantle. For accepting that Africa is stationary (that is, that primary convection beneath the continent is suppressed) and that all volcanism and uplift is essentially hotspot activity, it becomes possible to speculate on the nature of the plume convection system beneath.

As Thiessen (R.) *et al.* see it, there are some 30–40 hotspots/highspots within Africa's boundaries. That is, there are some 30–40 narrow zones of upwelling mantle material; and as what comes up must go down (well, some of it), there will be downwelling of mantle material approximately midway between hotspots. For a random arrangement of hotspots, this downwelling must occur along the edges of polygons (as viewed in plan), one of which surrounds each hotspot. In other words, there will be a pattern of polygonal convection cells, as seen from above, such that any points within a particular cell will be nearer to that cell's hotspot than to any other hotspot.

The construction of such polygons in a mathematical sense was first carried out by Thiessen (A.H.) in 1911 in connection with an analysis of rainfall data, whence, as widely used in computer cartography, they have come to be known as Thiessen polygons. Of more geophysical interest, however, is the fact that Richter and Parsons (*J. geophys. Res.* **80**, 2529; 1975) were able to generate polygonal cells in laboratory convection experiments involving oil layers heated from below in cases where the upper layer was stationary. Moreover, assuming the real-Earth plume cells to extend down only to the 700 km seismic discontinuity, the experimental and African polygons had the same width-depth ratios. Thus laboratory work and observationally-based hypothesis combine nicely to produce a consistent picture.

Except that Morgan's original plumes were deep-mantle plumes, possibly originating as far down as the core–mantle interface, whereas Thiessen's plumes appear to be an upper-mantle phenomenon only. Something seems to have been lost here without acknowledgment.

Peter J. Smith is a Reader in the Department of Earth Sciences at the Open University.

Atomic spectroscopy at Orsay

from Anne Thorne

Do you prefer to believe in 'action at a distance' or in 'hidden variables in quantum mechanics'? Put another way, if quantum mechanics affords a complete description of a system, it seems to be necessary to reject the idea of local causality — that is, the idea that the causes of effects are to be found inside the relevant backward light cone. Reviewing the present status of this old dilemma at a recent conference* J.S. Bell (CERN, Geneva) described a *Gedankexperiment* in which an excited atom in a state A undergoes the radiative cascade A→B→C, emitting a photon at each stage, where A and C have zero angular momentum and the same parity. Taking the particular case that the two photons are emitted in opposite directions, then if one is linearly polarised so also must the other be, with the electric vector in the same direction. Hence information from a perfectly efficient linear polariser on one side gives information about the signal from a second perfectly efficient linear polariser on the other side. Bell described attempts to translate *Gedanken* into reality, including a variant in which the angle between the polarisers is changed while the photons are actually in flight. Although the results so far seem to favour quantum mechanics rather than 'local causality', a sceptic can still find refuge in the allowances made for detector inefficiencies, finite acceptance angles, and so on. Attempts are now being made to close these loopholes and obtain what is expected to be incontrovertible evidence in favour of quantum mechanics. Meanwhile, most of us will continue to ignore the challenge of this intriguing problem by adopting the first of the three options Bell put forward — "Don't think about it at all"!

On the same day, nicely redressing the balance between the conceptual and the practical, H. Welling (Institute of Applied Physics, Hannover) discussed important new developments in tunable lasers. The spectral region over which tunable lasers can be operated is extending rapidly into both the ultraviolet and the infrared. Welling concentrated mainly on the solid state F-centre lasers for the infrared. In particular, the F_A and F_B colour centre lasers, made by doping crystals of the heavier alkali halides with lithium and sodium respectively, cover between them nearly all the spectral region between 0.8 and 1.9 μm. The power output is in the region of 100mW, with the laser running over two or three modes (2-3 GHz), but Welling suggested that the final resolution attainable should be of the order of 100kHz.

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*The 11th Conference of the European Group for Atomic Spectroscopy was held at Orsay on 10-13 July. The Organising Secretary was Professor B. Cagnac, ENS.

Anne Thorne is in the Department of Physics, Imperial College, London.

C. Cohen-Tannoudji (ENS, Paris) discussed several intriguing experiments with atoms in intense laser fields. For example, a gas can be radiatively cooled by momentum transfer between atoms and laser radiation detuned slightly from the absorption line centre, so that photons are absorbed only by atoms travelling towards them. The cooling after many such collisions is considerable: an effective temperature of as low as 0.2 K was mentioned. As another example, the energy shift of atoms in intense laser fields can be exploited for Doppler-free spectroscopy by choosing the intensity and the detuning of the laser to compensate the Doppler shift for atoms travelling in the forward direction. The Doppler shift for atoms travelling backwards is correspondingly increased resulting in a high degree of forward-backward asymmetry in the absorption line. Moreover, interpreting this same intensity shift as a change in potential, it can be seen that a high intensity gradient, as in a beam focused to a narrow waist, implies a force on the atom constraining it to the high field region. Two crossed lasers can thus provide a trap for neutral atoms, in which it should, among other things, be possible to observe a single atom over a finite period. This opens up the possibility of performing on neutral atoms the type of experiment (lifetime of metastable states, high precision hyperfine structure) that has recently been achieved with ion traps.

One particular set of experiments may be of interest to physicists outside the field of spectroscopy. These concern the measurement of the hyperfine structure of the artificial atoms muonium, μ^+e^-, and muonic helium, $\alpha\mu^-e^-$, a joint project between Heidelberg and Yale Universities, conducted at Los Alamos Scientific Laboratory. In the case of muonium, the ratio of the muon and proteon magnetic moments and the hyperfine splitting of the ground state are independently determined, and comparison of the latter with the theoretical value represents a clear test of the QED corrections. The muonic helium atom was aptly described as a "set of Dutch doll hydrogen atoms": the system electron plus pseudonucleus $\alpha\mu^-$ constitutes the outer hydrogen-like atom and the system $\mu^- + \alpha$ the inner one. ☐

International prostaglandin conference

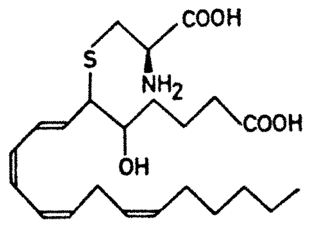
from Marie Foegh

ONE of the most exciting discoveries announced at the recent International Prostaglandin Conference* was the structure of slow-reacting substance (SRS) which has been determined by B. Samuelsson and colleagues at the Karolinska Institute, Stockholm (see Fig. 1). SRS-A (slow reacting substance of anaphylaxis), is one of the several mediators of anaphylaxis, and is of particular interest because it is synthesised *de novo* as a consequence of anaphylactic challenge and considered to be a major cause of bronchospasm during asthmatic attack. Despite much effort, the structure remained unsolved for many years.

SRS has now been identified as a novel cysteinyl derivative of arachidonic acid with a hydroxyl group at C-5 and three conjugated (Δ7, 9, 11) and one isolated (Δ14) double bond. The amino acid (cysteine) is linked as a thioether to C-6. These findings indicate the importance of the lipoxygenase pathway in its synthesis. Samuelsson introduced the term 'leukotriene' for these metabolites which are non-cyclised C_{20} carboxyl acids with one or two oxygen substitutes and conjugated double bonds. E.J. Corey (Harvard University), described routes of total synthesis to leukotriene.

SRS is a polar lipid-like substance. Arachidonic acid had previously been suggested as a precursor of SRS for various reasons and this is confirmed by the structure. The clinical implications of the newly-determined structure of SRS are apparent in immune responses of the immediate type. The non-steroidal anti-inflammatory drugs, such as aspirin, enhance SRS-A production *in vitro* by inhibiting the cyclo-oxygenase pathways of arachidonic acid metabolism. Aspirin is therefore expected to enhance SRS-A production *in vivo* and that may explain aspirin-sensitive asthma — an immediate anaphylactic reaction to aspirin and aspirin-like drugs observed in some asthmatic patients.

In clinical experience this pathological response occurs mainly in females. Falliers (*J. Allergy & clin. Immunol.* **52**, 141; 1973) has shown in a follow-up of 1,298 asthmatic patients who developed their asthma in childhood, that 1% of male patients and 4% of female patients had aspirin-sensitive asthma. These sex-differences may be related to the manner in which arachidonic acid is anabolised and catabolised. Sex differences in both platelet and vascular responses to arachidonic acid in animals were reported by P. Ramwell (Georgetown University, Washington, DC) where the male is more responsive than the female.



LEUKOTRIENE C
(SRS)

Fig. 1

J. Vane (Wellcome Research Laboratories, Beckenham, UK) presented further evidence for the hypothesis that the bleeding defect in the Greenland Eskimo is caused by an increased production of prostacyclin I_3 by vascular endothelium which inhibits platelet aggregation. An increase in production of a prostacyclin-like substance from arterial rings incubated with Δ5, 8, 11, 14, 17 eicosapentenoic acid has been demonstrated. Because of their fish diet, Greenland Eskimos have high plasma levels of eicosapentenoic acid which is the precursor of the three series of endoperoxides — prostaglandins, thromboxane and prostacyclin. In contrast to thromboxane A_2 produced from arachidonic acid, thromboxane A_3 has only a weak aggregating effect on platelets, while PGI_3 is a potent anti-aggregation substance. However, P. Needleman *et al.* (Washington University, St Louis), suggested an alternative explanation based on their observations that mammalian tissues (except for ram seminal vesicles) cyclised eicosapentenoic acid very poorly and that moreover, this acid had the same affinity as arachidonic acid for the cyclo-oxygenase enzyme. Furthermore, eicosapentenoic acid inhibited the cyclisation of arachidonate. Tissues and organs readily incorporated isotopic eicosapentenoic acid, like arachidonic acid, and it was as easily released as arachidonic acid following bradykinin stimulation. Needleman suggested that the bleeding defect in the Greenland Eskimo may be due to a decreased tendency for platelets to make thromboxane A_2 as a result of the presence of high levels of eicosapentenoic acid in plasma and platelet membrane inhibiting the cyclisation of arachidonate. Whatever the explanation, these observations raise the possibility of therapeutic intervention by diet.

Many papers dealt with the newly discovered prostacyclin. Because of its vasodilator and platelet anti-aggregating properties it has already been tested for treatment of peripheral artery diseases, and for its usefulness during haemoperfusion, haemodialysis and heart valve replacement.

In obstetrics, there have been further developments of prostaglandins of the E

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*The International Prostaglandin Conference was held in Washington DC on 27-31 May, and organised by P. Ramwell, B. Samuelsson, and R. Paoletti. The proceedings of the Conference will be available from Raven Press, New York.

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series, where 16-phenoxytetranor-PGE$_2$ methyl-sulphonyleamide (Schering, Pfizer), 16, 16-dimethyl *trans*Δ^2PGE$_1$ methylester (ONO) and 9-deoxo-16, 16-dimethyl-9-methylene PGE$_2$ (Upjohn) have been tested for use in both early (including menstrual regulation) and late abortion. The new compounds seem to produce fewer gastro-intestinal side effects and can be given intramuscularly, vaginally or orally. M. Bygdeman (Karolinska Institute, Stockholm) reported successful abortions in 10 of 12 women in the second trimester after oral doses of 25 mg of 9-deoxy-16, 16-dimethyl-9-methylene PGE$_2$ when given every third hour; the mean abortion time was 22 h.

The conference illustrated the general applicability of prostaglandins and related compounds to all aspects of medicine. Just as prostacyclin and thromboxane synthetase inhibitors are now being developed as anti-coagulants one can look forward to major efforts to develop specific lipoxygenase inhibitors and leukotriene C antagonists for clinical use. The development of radioimmunoassays for leukotriene C should be of considerable diagnostic value in immunological diseases. □

Marie Foegh is in the Department of Gynecology and Obstetrics, Herlev Hospital, University of Copenhagen.

Yes, NGF is an enzyme

IN a recent article in the News and Views section of this Journal, P. Calissano and R. Levi-Montalcini have questioned the validity of our work on the proteolytic activity of nerve growth factor (NGF), and in particular, its ability to activate plasminogen[1]. Because their article contains misinformation, and because several of our papers on the points raised by Calissano and Levi-Montalcini were overlooked, we wish to respond as follows.

In 1977 we observed that crude homogenates of mouse submandibular glands contain multiple molecular-weight forms of NGF[2]. The largest of these, with mass 116,000, was completely purified[2] and later shown to be a specific proteolytic enzyme that can activate plasminogen[3]. It is also the predominant form of NGF which is secreted in high concentrations in mouse saliva[4]. Calissano and Levi-Montalcini state that this form of NGF "... exhibits a protease activity with an almost evanescent degree of specificity for plasminogen ...". Yet, as shown in Figs 1, 3, and 4 in reference 3 there is nothing about the proteolytic activity of this species of NGF which indicates that it is transient. Rather, the enzyme activity is real, persistent, and highly specific. NGF is indeed much less active than urokinase in activating plasminogen, and we clearly pointed that out. We also stated that plasminogen might not be the natural (physiological) substrate for NGF — only that it was susceptible to activation by this protease[3].

With regard to the possibility that the protease associated with our NGF preparations might be a nonspecific contaminant[1], Fig. 4 in reference 3 presents strong evidence that this is not the case. This figure shows that when our preparations of the 116,000 molecular-weight form of NGF are examined by gel electrophoresis, all of the protein, all of the enzyme activity, and all of the immunoassayable NGF activity migrate with identical electrophoretic mobilities — as a single component.

More recent studies have revealed that this form of NGF exhibits some unusual features which are perhaps even more interesting than its plasminogen-activating property. For example, we have now shown that NGF, as isolated from mouse salivary glands, exists as a zymogen and further, that this zymogen is converted to fully active enzyme by autocatalytic self-activation[5]. The zymogen itself is largely, if not entirely, enzymically inactive. Only after autocatalytic activation is full enzyme activity expressed[5]. This finding is probably the strongest evidence that the protease activity of NGF is specific and that it does not stem from a contaminant.

With regard to the comment of Calissano and Levi-Montalcini that the NGF activity might be "stuck" nonspecifically to other proteins present in the salivary gland, we must point out that if this is so, then it is "stuck" extremely tightly, it is "stuck" in constant ratio from preparation to preparation, and it is also "stuck" as it is naturally secreted in saliva[4]. All of this goes to the heart of an elementary problem of protein chemistry; namely, what constitutes the definition of a true molecular species. All of our results outlined above suggest that the NGF-zymogen is such a species, and we know of no published evidence which indicates otherwise.

Finally, we should like to point out that of the several (at least six) forms of NGF present in salivary gland extracts, only the 116,000 molecular weight protein is stable[3]. All of the other forms are degradation products of this single species[2]. This includes the 2.5S-NGF (molecular weight 26,000) referred to by Calissano and Levi-Montalcini[1]. In fact, 2.5S-NGF accounts for less than 10% of the total NGF found in the salivary gland, and this minor component is the one which has been used in most experiments on NGF over the past twelve years.

The proteolytic activity of the 116,000 molecular weight form of NGF is a real property of this protein, and this fact, as well as the autocatalytic activation reaction, is going to have to be taken into account before the true physiological function of NGF can be understood.

MICHAEL YOUNG

Department of Biochemistry and Molecular Biology, University of Florida College of Medicine, Gainesville, Florida

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Sugars and intracellular recognition

from M. Geisow

STUDIES of the biosynthesis and structure of membrane glycoproteins have reached maturity in the past few years. The long-suspected role of sugars in cell-surface recognition processes has been established with the identification of specific receptors. However, the reason for the glycosylation of many soluble secreted proteins (plasma proteins and hormones for example) is still not at all clear. Do such conjugated sugars have analogous extracellular recognition functions? In 1965, while considering the selective export of secreted proteins, Eylar (*J. theoret. Biol.* **10**, 89) hypothesised that conjugated sugars formed part of a primitive mechanism of export from cells. This early idea was right to the extent that it predicted the existence of an export 'signal', but wrong in the sense that this signal has turned out to be a peptide rather than an oligosaccharide (see *News and Views* **272**, 308; 1978). In eukaryotic cells, newly synthesised proteins entering the lumen of the rough endoplasmic reticulum (RER) by virtue of this signal peptide must nevertheless possess further chemical or structural attributes which will determine their ultimate fate. For example, complex enzymic processing follows the biosynthesis of hormone precursors like ACTH/endorphin (Mains *et al. Proc. natn. Acad. Sci. U.S.A.* **74**, 3014; 1977). Furthermore, molecules within the ER/Golgi system, whether processed or not, need to be packaged in a different manner according to whether they are lysosomal, secreted or membrane proteins. Recent experimental work suggests the participation of conjugated carbohydrate in these intracellular recognition processes.

Melchers (*Biochemistry* **12**, 1471; 1973) found that the glycosylation inhibitor 2-deoxy-D-glucose prevented migration of

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M. Geisow is a member of the scientific staff at the National Institute for Medical Research, Mill Hill.

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newly-synthesised IgG1 from RER to smooth ER, although it is known that this sugar acts at a number of cellular sites. Subsequent studies have taken advantage of the more specific action of tunicamycin. This drug inhibits the attachment of N-acetyl glucosamine to a lipid carrier, dolichol phosphate, blocking the so called 'core glycosylation' of glycoproteins. Hickman *et al.* (*J. biol. Chem.* **252**,4402; 1977) supported Melcher's finding when they reported that secretion of newly-synthesised IgA and IgE from plasma cells was blocked by tunicamycin. The release of λ light chains (normally not glycosylated) was unaffected and there was only minor perturbation of protein synthesis.

Although intact, non-glycosylated immunoglobulins can be detected in quantity in tunicamycin-treated cells, other glycoproteins seem to be degraded intracellularly. Schwarz *et al.* (*J. Virol.* **19**, 782; 1977) found that tunicamycin prevented the formation of mature Semliki Forest virus and fowl plague virus (FPV). At least one of the virion proteins (the sugar-free precursors of FPV haemagglutinin) was subject to proteolysis, since the specific trypsin inhibitor TLCK was needed to detect the presence of this characteristic virus protein. Analogous intracellular proteolysis of non-glycosylated fibronectin (the extracellular matrix-forming protein) was reported by Olden *et al.* (*Cell* **13**, 461; 1978).

Related, but more subtle consequences of blocking glycosylation have been described for two soluble secreted proteins. Duksin and Bornstein (*J. biol. Chem.* **252**, 955; 1977) found that non-glycosylated procollagen was secreted normally by fibroblasts, but that a step in its extracellular conversion to collagen (removal of the 3-chain carboxyl-terminal extension) was impaired. Since the non-glycosylated procollagen was processed normally by extracts from untreated fibroblasts, the impairment was probably due to the absence of cosecreted processing enzyme. Loh and Gainer (*FEBS Lett.* **96**, 269; 1978) found that the biosynthesis of ACTH/endorphin precursor in frog pituitary was unaffected by tunicamycin. However, chase experiments indicated that the subsequent proteolytic processing of this precursor was quite different in tunicamycin-treated glands. The alterations in processing particularly affected the ACTH region of the molecule, which is close to a site of sugar attachment. The processed products were recovered in poor yield, suggesting that the precursor was exposed to nonspecific fragmentation.

Not all proteins derived from ER membrane-bound ribosomes are so markedly affected by tunicamycin. Leavitt *et al.* (*J. biol. Chem.* **252**, 9018; 1977) used tunicamycin to prevent the glycosylation of vesicular stomatitis virus and Sindbis virus proteins. The non-glycosylated versions were metabolically stable, but the proteins remained 'cryptic' — they could not be detected on the host cell surface at any stage during the process of infection. Moreover, the sugar-free virion proteins were insoluble in detergent, unlike the normal glycoproteins. The authors speculated that the altered intracellular migration and physical chemical properties of the proteins stemmed from an altered conformation of the non-glycosylated polypeptides.

Semliki Forest virus proteins also remain cryptic in tunicamycin-treated cells (Garoff & Schwarz *Nature* **274**, 487; 1978). In this case gross perturbation of conformation is unlikely, since the non-glycosylated proteins are processed correctly by host cell proteases and inserted correctly into the ER membrane. Ovalbumin is one soluble secreted protein whose biosynthesis and release appears to be unaffected by tunicamycin-treated hen oviduct cells (Keller & Swank *Biophys. Biochem. Res. Commun.* **85**, 72; 1978).

An unattributable item of biochemical wisdom alleges that attachment of sugar moieties to glycoproteins confers a degree of immunity to proteases and that this occurs as much by design as by chance. In the ACTH/endorphin system already referred to, the carbohydrate on the bovine precursor molecule (Nakanishi *et al.* *Nature* **278**, 423; 1979) is very probably close to such a proteolytic cleavage site. Absence of carbohydrate sterically placed to shield susceptible peptides might well explain the degradation of certain non-glycosylated molecules. In fact, this explanation is invoked by some authors. However, an extension of this argument seems worth mentioning here, since it seems not to have previously been considered. By some device secretory material, processing enzymes and the complement of lysosomal enzymes must be segregated and separately packaged in the ER/Golgi system.

The majority, and possibly all, of the lysosomal proteins are glycoproteins. One in particular, β-glucuronidase, is known to be attached to the inner surface of the lysosomal membrane. Moreover, lysosomal proteins like β-glucuronidase in the extracellular space seem to be efficiently taken up into cells by way of a receptor-mediated event which recognises covalently-linked hexosephosphate (see *News and Views* **269**, 288; 1977).

These studies may be leading to the establishment of phosphorylhexose as an intracellular recognition signal. What happens to newly-synthesised, non-glycosylated lysosomal enzymes when secretory cells are treated with glycosylation inhibitors? Might they escape such a recognition system and enter an inappropriate compartment — a secretion granule for instance?

This line of argument leads full circle to Eylar's original hypothesis about the intracellular recognition role of conjugated sugars. Sugars do not specify export as such, but if they represent 'routing' signals within the ER/Golgi, then the diverse effects of tunicamycin might be more readily explained. Exploration of this idea will require experiments which trace normal and carbohydrate-free versions of membrane, secreted and lysosomal proteins through the cell. □

Electronic dimples explained

from P. V.E. McClintock

EARLIER this year M. Wanner and P. Leiderer reported (*Phys. Rev. Lett.* **42**, 315; 1979) that they had observed an intriguing two-dimensional crystal lattice of electron filled dimples at the interface of a phase separated ^{3}He-^{4}He mixture. This unexpected experimental result, which was obtained at the Technical University of München, has now received a fairly complete explanation from H. Ikezi of Bell Laboratories, who describes his calculations in a recent issue of the same journal (*Phys. Rev. Lett.* **42**, 1688; 1979).

The two dimensional (2-D) layer of electrons which can be floated on the free surface of liquid helium has been studied in considerable detail: it is believed to take the form of a 2-D crystal lattice of the type first proposed by Wigner (*Trans. Faraday Soc.* **34**, 678; 1938). In practice an electric field must be applied, pushing the electrons on to the surface, and depressing the centre of the liquid slightly. The individual electrons, which are highly mobile parallel to the surface, are thus prevented from spreading under their mutual Coulomb repulsion. If this electric field is made large enough (a few kV cm^{-1}), however, it is found experimentally that the surface eventually becomes unstable. The charge suddenly penetrates into the liquid, and it finally gets caught by the lower of the two electrodes providing the electric field. Studies of this instability provide information about the electron lattice and about surface waves on the liquid. Some quite detailed investigations have already been performed including, particularly, a beautiful cinematographic film of the onset of the phenomenon taken by Volodin, Khaĭkin and Edel'man (*J. exp.theor.Phys. Lett.* **26**, 543; 1977) and shown at the 15th International Conference on Low Temperature Physics at Grenoble in 1978.

Very similar considerations apply to the interfacial boundary which occurs where liquid ^{3}He floats on top of a dilute ^{3}He-^{4}He solution, but this system has only recently come to be studied in any detail. Again, the

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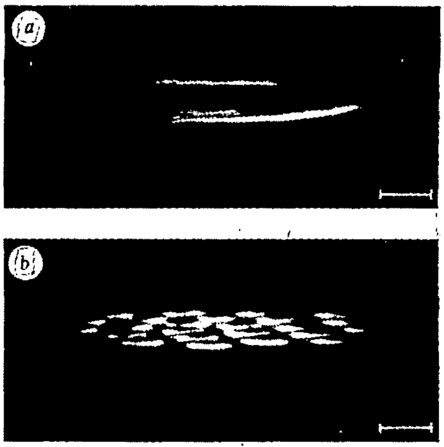

Fig. 1 Photographs of the deformation of the charged liquid ^{3}He/^{4}He interface at a temperature of 0.567 K, observed from below at an angle of 6°. *a*, For an electric field E = 1120 V cm^{-1} a single large circular indendation is produced, the elliptical bright lines indicating its rim. *b*, For E = 1160 V cm^{-1} the large indentation has spontaneously deformed into an array of much smaller circular dimples. The scale bar in each case in 1 mm.

interface can be charged with electrons and can then be depressed slightly by an electric field. Wanner and Leiderer's experimental arrangement allowed them to create ripples on the charged interface and to study the frequency-wavelength relationship (dispersion relation) using an optical technique. As anticipated theoretically they found that, for large electron densities and electric fields, the modes 'softened' — their frequencies become less for any given wavelength. This effect is a consequence of a reduction in the net restoring force for a displacement of the interface, caused by a rearrangement of electrons in the vicinity of the ripple. For very large fields, the frequency approaches zero and the whole arrangement becomes unstable, with charge breakthrough eventually occurring.

What Wanner and Leiderer found, however, was that a series of macroscopic dimples sometimes formed in fields quite close to that producing breakthrough. Their striking photographs are reproduced in Fig. 1. In (*a*) the surface is seen to be depressed in a single large diameter indentation; but, with quite a small increase in the electric field, it spontaneously breaks up into a lattice of small indentations, as shown in (*b*). The indentations were typically 1 mm apart, forming what appeared to be a hexagonal lattice (although the oblique viewing angle made exact identification of the structure rather difficult). Since each dimple was calculated to contain about 10^6 electrons, it was not at all surprising that they should try to repel each other in order to minimise their Coulomb energy and, in so doing, form a macroscopic 2-D Wigner lattice. The authors added the intriguing thought that the electrons in each dimple would presumably also be arranged in a 2-D lattice, but with a lattice constant some three orders of magnitude smaller than that of the dimple lattice. What was not entirely obvious, however, was why any dimples should be produced in the first place.

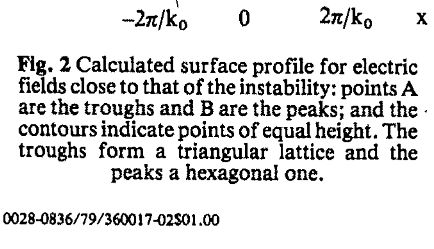

Fig. 2 Calculated surface profile for electric fields close to that of the instability: points A are the troughs and B are the peaks; and the contours indicate points of equal height. The troughs form a triangular lattice and the peaks a hexagonal one.

Ikezi has now produced the answer. He has done so by extending a theoretical approach originally proposed by L.P. Gor'kov and D.M. Chernikova (*Soviet Phys. Dokl.* **21**, 328; 1976), and has developed a theory of the surface ripples which includes nonlinear terms. The latter turn out to be of great importance near the instability. Amongst the most interesting conclusions to emerge from his calculation is that, under certain conditions, his six coupled equations lead to solutions representing a surface profile which is both stable and static. It forms the lattice shown in Fig. 2. As far as one can tell, this is in excellent agreement with the dimple structure observed in the actual experiments (Fig. 1*b*).

Ikezi points out that both shear and compressional waves should propagate through the ^{3}He-^{4}He dimple lattice; and that similar phenomena should also be observable on a free surface of superfluid ^{4}He. He predicts that the frequencies of these lattice waves should be typically a few Hertz, in which case they should not be too difficult to observe. It will be interesting to see whether he is right. □

P.V.E. McClintock is a Senior Lecturer in the Physics Department at the University of Lancaster.

Are supergroups the next step for electro-weak interactions?

from J.G. Taylor

IN THE past year impressive experimental confirmation has been obtained for the so-called electro-weak theory (EW) of Salam and Weinberg which unifies the electromagnetic and weak interactions under the gauge group SU(2) × U(1).

There is still a discrepancy between the Russian experiment on parity violation at Novosibirsk and an almost identical experiment conducted at Oxford [1]; the former agrees remarkably well with the predictions of the Salam–Weinberg model, in complete disagreement with the null result obtained by the latter. In spite of this there is almost universal acceptance of the electro-weak theory.

However, there are still unsatisfactory features of the model. Thus the predicted intermediate vector bosons, (IVBs) of mass about 80 GeV, have still to be seen; though they should be created around 1981 at the p$\bar{\text{p}}$ facilities now being built. There is also considerable freedom in the mechanism as to how the IVBs get their masses; even the favoured one of spontaneous symmetry breaking (SSB) has great latitude in its detailed operation. Furthermore the EW theory is not really a unified theory; the relative strengths of the interaction in the SU(2) and U(1) parts of the gauge group has to be obtained from experiment. The parameter measuring this strength, the Weinberg θ_W, has now experimentally settled down very close to the value θ_W = 30° (ref.2) as given at the CERN EPS meeting in Geneva at the end of June.

Y. Ne'eman [3]; and D. Fairlie [4] both independently noticed that the smallest group containing SU(2) × U(1) and which should require θ_W to be 30° is the supergroup SU(2|1). This latter has as generators of its group algebra the four week isopin and weak hypercharge generators of SU(2) and U(1) and in addition four anti-commuting generators. These additional generators have therefore the characteristic of fermions, and should correspond to spin half integer associated gauge fields.

Various attempts [3-6] have been made to construct a satisfactory gauge field theory of SU(2|1). None of these has been able to overcome completely the difficulty that since the symmetry group SU(2|1) is not combined with the Poincaré group symmetry of space–time the gauge fields of SU(2|1) will all be vector fields. If they have Fermi statistics, as should those which gauge the anti-commuting generators of SU(2|1), they will therefore violate the spin-statistics theorem (which only allows integer spin fields to have commutation and not anti-commutation rules). Since the

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spin-statistics theorem can be deduced from very general field-theoretical principles such a violation is not to be accepted.

Some attempts[3,5] have been made to avoid this difficulty by introducing anti-commuting supergroup parameters related to these odd generators. However the resulting dynamical theories seem to possess ghosts and other undesirable features. An alternative approach is to construct a dynamical theory which has classical fields so chosen as to be invariant only under infinitesimal variations of the group SU (2|1). The condition of infinitesimal invariance leads to a sensible physical theory and also specifies the mechanism of SSB with no further latitude. Furthermore the theory avoids the problem of wrong-statistics gauge fields by no longer allowing arbitrary gauge invariance. Only the original electro-weak symmetry group SU(2) × U(1) is full gauged; the wrong-statistics gauge fields are not required.

Such an approach does not follow the tradition of gauging all possible symmetry groups. However it cannot be dismissed lightly since it does completely unify electromagnetism and weak interactions with Weinberg angle θ_W of 30°, as well as predicting the masses of the IVBs to have the same value as the original Weinberg–Salam theory. Furthermore it predicts an associated neutral scalar (Higgs) boson to exist with mass exactly twice that of the charged IVBs. Such a prediction may also be tested at the future $p\bar{p}$ facility, though with greater difficulty than for the IVBs due to the rather weak coupling of the Higgs boson to normal matter. Another prediction which can also be tested in about 1981 is that no lepton like the electron or muon can have mass larger than about 54 GeV.

The above theory can also be extended to include strong interactions. Quarks can be fitted into the four-dimensional representation of SU (2|1) (as the leptons can into the three-dimensional one), and their colour properties as triplets under the colour gauge group SU $(3)_{\text{colour}}$ included by extending SU (2|1) to SU (5|1). Each generation of leptons and quarks (the first being e, ν_e, u, d, the second μ, ν_μ, c, s, the third τ, ν_τ, t and b) can be fitted into the 16-dimensional representation of SU (S|1), which is then reducible to the 15-dimensional representation of SU (5). This latter group has been favoured as the smallest which can contain $SU(2) \times U(1) \times SU(3)_{\text{colour}}$. SU (S|1) may thus be considered as the smallest supergroup which can properly unify the strong, electromagnetic and weak interactions.

As for SU (5) (ref. 7), SU (5|1) also has difficulties over the value of θ_W and proton stability. Latest experimental values of θ_W are nearly incompatible with the known proton lifetime in SU (5), so that a larger group may be needed. SU (S|1) has also greater latitude in the Higgs boson sector than for SU (2|1), though a neutral Higgs boson mass of twice that of the charged IVB is still predicted, along with numerous other similar particles.

Besides these predictions an interesting feature of the theory is its requirement[3,6] of extra dimensions whose only evidence is in the Higgs boson structure. Thus the theory is of Kaluza–Klein type. However the extra dimensions are required to be time-like instead of the traditional space-like ones heretofore. Each new generation of particles requires a further two time-like dimensions for its description; at present there are therefore six extra time-like dimensions alongside our present one and the associated three space-like dimensions. These extra dimensions carry only the symmetry of the theory, but are otherwise trivial. Verification in the 1980s of the predictions of the theory will give support to the existence of these extra dimensions; a very exciting possibility to look forward to.

J.G. Taylor is Professor of Mathematics at King's College, London.

NATURE

A hundred years ago

Radiant Matter proceeds in straight Lines

The radiant matter whose impact on the glass causes an evolution of light, absolutely refuses to turn a corner. Here is a V-shaped tube (Fig.6), a pole being at each extremity. The pole at the right side (*a*) being negative, you see that the whole of the right arm is flooded with green light, but at the bottom it stops sharply and will not turn the corner to get into the left side. When I reverse the current and make the left pole negative, the green changes to the left side, always following the negative pole and leaving the positive side with scarcely any luminosity.

In the ordinary phenomena exhibited by vacuum tubes — phenomena with which we are all familiar — it is customary, in order to bring out the striking contrasts of colour, to bend the tubes into very elaborate designs. The luminosity caused by the phosphorescence of the residual gas follows all the convolutions into which skilful glass-blowers can manage to twist the glass. The negative pole being at one and the positive pole at the other, the luminous phenomena seem to depend more on the positive than on the negative at the ordinary exhaustion hitherto used to get the best phenomena of vacuum tubes. But at a very high exhaustion the phenomena noticed in ordinary vaccum tubes when the induction spark passes through them — an appearance of cloudy luminosity and of stratifications — disappear entirely. No cloud or fog whatever is seen in the body of the tube, and with such a vacuum as I am working with in these experiments, the only light observed is that from the phosphorescent surface of the glass. I have here two bulbs (Fig.7), alike in shape and positionof poles, the only difference being that one is at an exhaustion as will give the ordinary luminous phenomena — whilst the other is exhausted to about the millionth of an atmosphere. I will first connect the moderately exhausted bulb (A) with the induction-coil, and retaining the pole at one side (*a*) always negative, I will put the positive wire successively to the other poles with which the bulb is furnished. You see that as I change the position of the positive pole, the line of violet light joining the two poles changes, the electric current always choosing the shortest path between the two poles, and moving about the bulb as I alter the position of the wires.

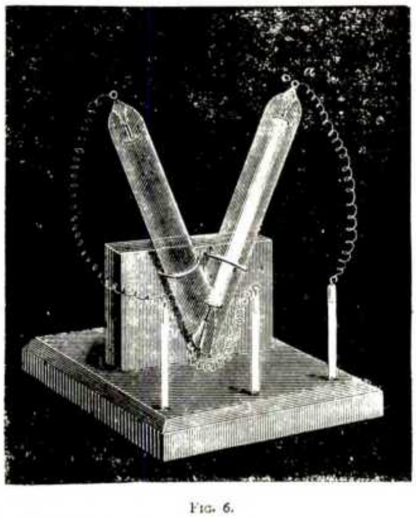

Fig. 6.

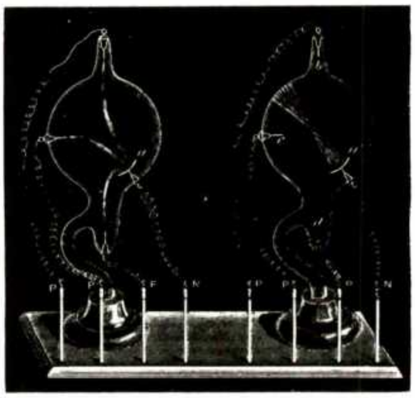

Fig. 7.

This, then, is the kind of phenomenon we get in ordinary exhaustions. I will now try the same experiment with a bulb (B) that is very highly exhausted, and as before, will make the side pole *(a)* the negative, the top pole *(b)* being positive. Notice how widely different is the appearance from that shown by the last bulb. The negative pole is in the form of a shallow cup. The molecular rays from the cup cross in the centre of the bulb, and thence diverging fall on the opposite side and produce a circular patch of green phosphorescent light. As I turn the bulb round you will be able to see the green patch on the glass. Now observe, I remove the positive wire from the top, and connect it with the side pole (*c*). The green patch from the divergent negative focus is there still. I now make the lowest pole (*d*) positive, and the green patch remains where it was at first, unchanged in position or intensity.

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review article

Radioisotope clocks in archaeology

R. E. M. Hedges
Research Laboratory for Archaeology and the History of Art, 6 Keble Road, Oxford, UK

Any absolute dating method requires some form of clock. This review considers those methods in archaeology in which the clock is the rate of disintegration of a radioactive nucleus.

UNTIL about 30 years ago, absolute dating in archaeology was restricted to the interpretation of surviving king lists and the like, while most dating was estimated from stratigraphic or cultural sequences, and their relationship to geological processes. The incorporation of radiocarbon dating has caused a sometimes dramatic[1] modification of accepted ideas, but is now regarded as an essential foundation to archaeological interpretation. Since the discovery of radiocarbon, other dating methods, with application in different circumstances, have been developed; and in the past two years new methods of detecting trace quantities of rare isotopes promise to extend the range of possibilities.

For a given isotope, its rate of disintegration, with its exponential decay law, is essentially completely independent of environmental influences. In this respect it contrasts strongly with the use, for example, of chemical clocks (such as the rate of racemisation of amino acids in degrading bone collagen, or the rate of weathering of flint). However, environment is not entirely without effect. Conditions at the 'start' of the clock must often be inferred, while, of course, there must be a connection between the 'start' and the event being dated. In fact the main challenge of dating lies in the precise articulation of these features. The difficulties of particular methods are considered below, but the example of radiocarbon illustrates the point. For radiocarbon dating, the 'start' of the clock occurs at the death of the organism which is destined to become the sample to be dated. At death, ^{14}C is no longer exchanged with its environment (that is food and atmospheric CO_2), and so the level of ^{14}C decays from its original, constant value. The original level can only be inferred from a consideration of all the processes forming and diluting ^{14}C in the relevant part of the environment, or alternatively by calibration against known-age samples. Biological fractionation and contamination must also be taken into account. With a figure for the original level, and a measurement of the present day level, the time elapsed for exponential decay is simply given by

$$t = 3.32\lambda \log_{10}\left(\frac{L_0}{L_t}\right)$$

where λ is the half life, L_0 the ^{14}C level inferred at the start, and L_t that measured. For ^{14}C the 'start' is comparatively well defined, although the death of a tree, for example, has to be more carefully formulated to apply to metabolic processes of, say, cellulose in an individual tree ring. Archaeologically, the felling of the tree may be regarded as the event to be dated, which does not necessarily correspond to the same thing at all. Also the possible re-use of a timber from an earlier structure makes the purely archaeological interpretation of a radiocarbon date that much more involved.

Archaeology is primarily concerned with the cultural development of man, but this is so closely bound up with man's biological and ecological development that the dating of the latter aspects is of equal importance. Thus while a substantial fraction of archaeological work is concerned with the past 10,000–15,000 yr, dating techniques extending to beyond 5 Myr are necessary. The accuracy of dating depends on the complexity of the information to be resolved. Rapid cultural development may require a date to within ±20 yr, which, at, say, 6,000 yr BP, corresponds to ±0.3% in the date. Beyond 30,000 yr, where radiocarbon dating is difficult to apply, sites are sparse and dates relatively few. Beyond 100,000 yr the rate of change is likely to follow the rate of environmental, such as climatic, change. The study of environmental change is not only important in its own right, but is also necessary for the proper evaluation of the dating techniques themselves. For example, the change in global ice volume can be followed from oxygen stable isotope measurements in deep sea cores[2], the sudden release of 'aged' CO_2 dissolved in retreating glaciers can then cause perturbations of the ambient ^{14}C level in the biosphere.

General approaches

The methods considered here can be grouped into three categories: (1) The decrease in concentration of a radioisotope from a presumed initial level, or the reciprocal build-up of a stable daughter product; (2) where a chain of radioactive decays exists, the degree to which the members of the chain are restored to equilibrium following some external initial perturbation; (3) the integration of some local radioactive process by the sample material. The total can then be compared with the value of the local flux, which is usually due to the presence of radioisotopes and remains approximately constant in time.

Methods (1) and (2) technically depend on the need to make accurate measurements of concentrations of radioisotopes or their daughter products, while (3) depends on exploiting a reliable integrating process. In all these cases assumptions must be made about initial conditions, and sometimes about the subsequent history. Often it is not possible to test such assumptions adequately unless a comparison, using a dating method dependent on quite unrelated assumptions, is available. The overlap between different methods has up to now been rather sporadic (mainly owing to differing time scales or materials). The recent developments in ultra-sensitive detection of specific isotopes[3], for example, ^{10}Be, ^{14}C, ^{36}Cl, have given a new impetus to the field, and the availability of new methods, with new time scales, should help considerably to bring about such cross-checking.

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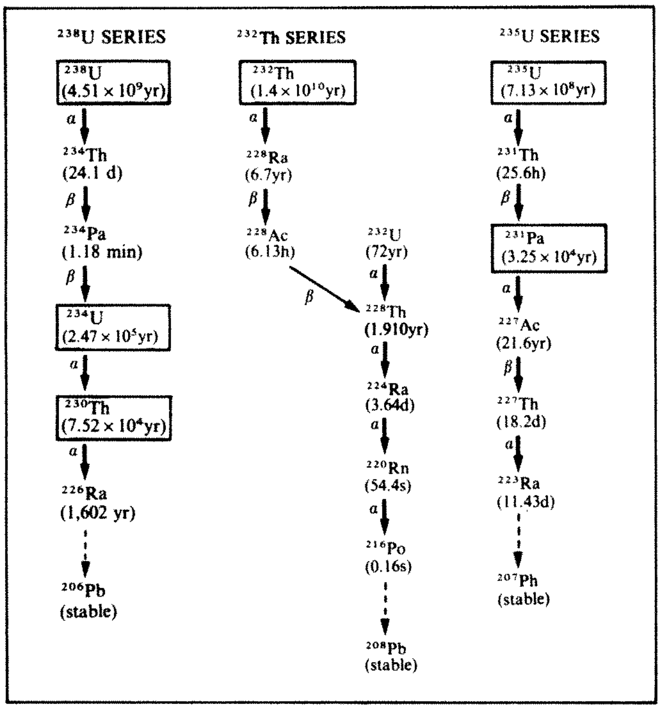

Fig. 1 Uranium and thorium decay series, indicating mode of decay and half life for the nuclides. Long-lived isotopes for dating are enclosed in boxes.

Specific techniques

For convenience we start with the abundant radioisotopes (^{40}K, Th and U), pass to the rare radioisotopes (^{14}C, ^{10}Be, ^{26}Al, ^{32}Si, ^{36}Cl, ^{41}Ca, ^{53}Mn), and finally consider radiation integration techniques (fission track dating, thermoluminescence and related techniques).

K/Ar: ^{40}K occurs with a natural abundance of 0.012% relative to ^{39}K, and a half life of 1.3×10^9yr. A small fraction decays to ^{40}Ar, so that a measurement of the accumulated ^{40}Ar, together with the quantity of parent ^{40}K (effectively constant over a few Myr) gives the age directly by integrating the exponential decay. This method is primarily applicable to volcanically formed rock such as tuff or pumice. Of course, the rock must not incorporate any additional ^{40}Ar, for example from the atmosphere, and no radiogenic ^{40}Ar must be lost from the rock. Note also that the younger the rock the less radiogenic Ar, and the greater the possibility of contamination and difficulty of measurement. Typically, rocks younger than 50,000–100,000 yr become very hard to date reliably. (The basic method and literature are well covered in ref. 4.)

More recently, the measurement of ^{40}Ar and ^{40}K has been modified in a way which can take account of material in which ^{40}Ar is lost or modified by weathering or reworking. (For reviews, see refs 5 and 6.) In this technique, ^{39}K (which is in constant ratio to ^{40}K) is measured by converting it partially to ^{39}Ar by subjecting the sample to neutron activation. The ^{39}Ar so formed is in a similar micro-environment to that of the entrapped radiogenic ^{40}Ar. On heating the activated sample in incremental steps the ratio of ^{39}Ar/^{40}Ar can be measured mass spectrometrically, and its degree of constancy indicates the integrity of the material. This is particularly important for the younger samples relevant to archaeology. Measurement of ^{36}Ar allows the proportion of atmospheric Ar contamination to be estimated, and correction for Ca-derived Ar (in the neutron activation) must also be made, especially for young samples.

The necessary assumptions in K/Ar dating are concerned with the particular samples rather than with initial conditions. The ^{39}Ar/^{40}Ar method allows the validity of these assumptions to be tested for a given case, but real confidence, especially for more recent material, comes from independent dating. Very satisfactory agreement between K/Ar and fission track dating on the age of the KBS tuff from Lake Rudolph[7,8] has been obtained. The K/Ar method has been most useful in archaeology in dating the stratigraphy for the finds of hominids. Since it is the rock that is being dated, a stratigraphic relationship must be used to provide the archaeological date.

U: There are several somewhat different methods of uranium series dating. (A general review has been given by Ku[9].) In the decay of ^{232}Th, or ^{235,238}U, a series of daughter products is encountered with a wide range of halflives. In a closed system each daughter would be in 'secular' equilibrium, but because the geochemistry of some of the longer-lived daughters differs greatly a chemical separation in the environment takes place. The degree of restoration of equilibrium, whose rate is governed by the various half lives involved, can be measured. For example, in the decay of ^{238}U, the first long half life (see Fig. 1) of a non-U member is ^{230}Th ($\lambda = 7.5 \times 10^4$ yr), and this isotope is rapidly precipitated from an aqueous environment. The parent ^{238}U will not then be in equilibrium with its daughters in natural waters. If the uranium is now co-precipitated with, say, calcium as calcite, there will be a build-up of ^{230}Th from a null concentration from ^{234}U and ^{238}U, at a rate in accordance with their half lives, until secular equilibrium is regained. Traces of ^{230}Th from detrital sources which might be incorporated can be estimated, to first order, from a measurement of the ^{232}Th present, if this is small. Often such detritus can be chemically distinguished from the calcite deposit. Provided the initial concentration of ^{230}Th is small, a measurement of the ^{230}Th component of the calcite, together with that of ^{234,238}U, allows the age of the calcite since it was formed to be computed. For suitable uranium concentrations this method is applicable to ages in the 50,000–250,000-yr range.

A major application is to carbonates, where the assumptions required are a specification of the isotopic composition during formation of the carbonate precipitate, and the maintenance of a closed system afterwards while equilibrium is being restored. In most cases, it is the isolation of the system that causes more problems. Thus corals seem to be satisfactory subjects[10] while fossil shells do not[11]. There has been increasing interest in the dating of speleothems (cave deposits), an application with a more direct contact with archaeological interests. Precipitated calcite in these environments seems to constitute a satisfactory closed system, and difficulties over the initial isotopic composition (such as ^{230}Th from dust) are not usually serious. The archaeological applications of speleothem dating by the uranium series method will be reviewed elsewhere[12]. Schwartz *et al.*[13] have previously described an application to a specific archaeological site.

Attempts have been made to extend the method to date bone[14], which strongly accumulates uranium by ion exchange during burial. However, the difficulty of taking into quantitative account the extent to which bone is an open system renders this approach hazardous, and it has not been generally pursued, despite promising results. As one of the extremely few methods that deals with the original archaeological material on a time scale beyond radiocarbon, this application deserves more attention.

^{210}Pb: Although a member of the uranium series, the 22-yr half life of ^{210}Pb puts it into a different sphere of application. Disequilibrium for ^{210}Pb arises from two causes; in terrestrial environments escape to the atmosphere of ^{222}Rn in the decay of ^{238}U brings about a precipitation of unsupported ^{210}Pb as its daughter, and the decay of the ^{210}Pb which finds its way, for example, into lake sediments enables recent stratigraphy[15] to be dated. Archaeological applications to dating using a 200-yr time scale are limited, but one use is in the determination of authenticity. Here disequilibrium is produced in a lead containing artefact during the lead extraction process, so that initially ^{210}Pb is in excess, unsupported by ^{226}Ra. This method has been applied to the dating of lead pigments[16].

The isotopes so far discussed have a terrestrial origin, with parents of very long half lives. The isotopes next to be considered have a cosmic origin[17], and are formed in minute amounts. For example, the world inventory of ^{26}Al is estimated at only 1 ton. For these isotopes especially, the use of accelerators for their detection[3] promises to bring major changes to our present knowledge, although the isotopes considered here have all been detected by conventional methods with one exception (^{41}Ca). A brief survey of the new possibilities has been made by Arnold[18].

^{14}C: A comprehensive review of ^{14}C dating in archaeology has been given by Aitken[19]; the subject is too vast to attempt here, but it is worh indicating the similarities and differences between ^{14}C and the less-studied isotopes.

First, ^{14}C has some extremely fortunate properties which make it eminently suitable for archaeological dating. These are: a convenient half life (5,730 yr); the ability to equilibrate with the biosphere and so have a direct interaction with man's activities; and a uniform global distribution arising from its equilibration with dissolved oceanic CO_2. Furthermore, ^{14}C is present in sufficient abundance to provide adequate samples for its measurement ($^{14}C/^{12}C = 10^{-12}$ for recent material). ^{14}C is formed by cosmic rays through the (n, p) reaction on atmospheric ^{14}N, unlike the other isotopes which are predominantly derived from spallation processes. Again unlike the other isotopes (except ^{36}Cl), it is oxidized to a stable gas molecule, CO_2, which equilibrates with the major carbon reservoir of the Earth, the oceans. The level of ^{14}C in the atmosphere, which is responsible for the level in vegetation and thence in such archaeological materials as charcoal or bone, is maintained by a great number of interrelated processes[20]. The formation rate[21,17] depends strongly on the degree of shielding of galactic cosmic rays both by the Earth's magnetic field and by that of the Sun, both of which vary with time. Because there is no energy threshold for ^{14}C formation (unlike the other isotopes) the formation rate is also more sensitive to bursts of low-energy protons during solar flares. Equilibration with the ocean (or rather the surface mixing layers) has a fractionating effect, which is temperature dependent, and is therefore influenced by global and local climate. Mixing rates with the deep ocean, which is the main reservoir, are not short compared with the half life, and provide a further source of variation. The imprisonment of dissolved CO_2 in glaciers and ice caps has already been mentioned. The well known calibration of ^{14}C levels[19,22–24], based on an independent dendrochronology of samples, shows that for the past 7,000 yr the atmospheric level has varied by <10%. However, the variation actually found shows a very interesting relationship to known changes in the geomagnetic field[25], as well as shorter-term changes related to solar activity[26]. From the immediate archaeological viewpoint these world-wide variations[27], enable calendar dates to be derived from the measured residual ^{14}C level in a sample. We still need to take account of fractionation in the metabolic processes which incorporate the ^{14}C in the sample, but this can be done through a measurement of the parallel fractionation in the stable isotope $^{13}C/^{12}C$ ratio for the same sample. In some cases a rapid fluctuation in atmospheric ^{14}C level can lead to ambiguous calendar dates, but this is fortunately rare for the period studied. However, it does increase the range of error for a calibrated date. Where a sequence of accurate ^{14}C measurements can be made, as in individual growth rings, a very accurate calendar date may be possible by fitting to the appropriate deviation on the calibration curve ('wiggle matching'). For much earlier times, calibration must await an independent, high resolution, alternative dating. There is some indication that ^{14}C atmospheric levels have remained fairly constant for the past 30,000 yr[28], and some evidence that cosmic ray production rates have not varied greatly (less than a factor of 10% on average over the past 10 Myr[17,29]).

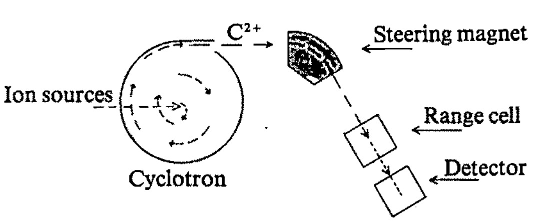

Fig. 2 High-energy mass spectrometry for the detection of ^{14}C using a cyclotron. The ion source may be external or internal. N^{2+} is removed at the range cell. The detector measures the energy loss rate and total energy of each particle.

Measurement of ^{14}C residual levels has hitherto been made by counting the ^{14}C β-decay rate. This has necessitated large samples (1–5 g), and has placed a limit of 40,000 yr on the age range (extended to 70,000 yr in some special cases). The accuracy of measurement now possible is ±0.2% corresponding to ±16 yr. The typical archaeological sample is rarely dated to this accuracy, however, mainly because of the degree of chemical and mechanical contamination while buried.

An alternative method of measuring ^{14}C, by counting ions rather than nuclear distintengrations, enables smaller samples to be used (by a factor of 10^4). This allows new types of material to be dated; for example, iron artefacts containing dissolved carbon, or single bones containing residual collagen, or individual surviving seeds. Samples can be stringently tested and purified to avoid chemical contamination. Also there is much greater choice available in selecting samples. The very low backgrounds in measurements by ion counting technique enables very much older material, perhaps to 100,000 yr, to be dated. All these methods (Figs 2 and 3) are based on a form of mass spectrometry, and they accelerate the ions up to high energies (>1 MeV AMU^{-1}) in order to take advantage of the nuclear techniques of particle identification. For example, at such energies ions of the same mass, charge and energy, such as $^{14}C^{4+}$ and $^{14}N^{4+}$, interact differently with matter, losing energy at different rates, and they can therefore be distinguished. Both cyclotrons[30] and tandem electrostatic accelerators[31–33] have been used (see also ref. 3). The Cyclotron provides a mass spectrometer with a very high rejection ratio; the tandem electrostatic accelerator, by virtue of the charge changing interaction at the stripper, can qualitatively reject many particles (for example, molecular ions). The relative merits of each approach depend on many technical issues, including the particular isotope studied, but those groups actively involved in the development of ^{14}C dating are all using tandem accelerators. It remains to be seen whether the mass spectrometric method can equal the radioactive counter method in accuracy.

^{10}Be: The ^{10}Be isotope, as with ^{26}Al and ^{32}Si, is produced in the upper atmosphere and circulates as an aerosol. Mixing with the troposphere is unevenly distributed throughout the Earth, and the isotope is rapidly precipitated from the troposphere in rain. There is a strong latitudinal variation, reflecting precipitation and mixing rates, and also, at least for ^{32}Si, a seasonal variation. The quantities of these isotopes reaching the Earth should respond much more sensitively to rapid fluctuations in the cosmic ray flux, since their residence time in the atmosphere is so much less than ^{14}C (which equilibrates with the ocean surface). ^{10}Be has been measured in cores from Antarctic ice[34,35] and also in deep sea sediments[36].

^{10}Be has a half life of 1.5 Myr, and so would provide a suitable time scale to date Pliocence–Pleistocene events. From the strictly archaeological point of view there seems little prospect of any direct connection between an archaeologically significant event and the 'start' of the Be clock, except through the dating of sediments. However, the accurate calibration of sediment cores allows one to calibrate other important stratigraphic measurements, such as changes in fauna and flora, in $^{16}O/^{18}O$ values, magnetic field reversals and changes in inclination and declination. The dating of ocean cores is still at a very early stage[38,39] and is partly hampered by the need to study the authigenic Be-containing materials of such cores. This implies careful selection of material, and so limits the sample size. Although the way in which ^{10}Be is deposited in cores is not well understood, good agreement has been obtained between magnetic reversal

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dates and ^{10}Be ages measured on the assumption of a constant formation rate, but with a sedimentation rate adjusted to produce a consistent variation of date with depth[39].

As for ^{14}C, high-energy mass spectrometry is a suitable technique to apply to ^{10}Be measurement, thus overcoming the main difficulty of the quantity of sample required. By this technique ^{10}Be has been measured in natural samples. In particular, Raisbeck has used a cyclotron to measure ^{10}Be in Antarctic ice cores[35], and more recently, in the ocean surface water[37]. With a measured average of about 700 atoms ml^{-1}, even the atom counting method required the sampling of several cubic meters of water. With the availability of this technique the study of ^{10}Be geochemistry should now develop rapidly. The total residence time of ^{10}Be in the oceans before sedimentation, is particularly important, since this will determine the frequency response to its formation rate of the sedimentary record. For example, the data of Tanaka[39], on two cores, suggest that long-term variations (0.7 Myr time scale) are $<\pm 10\%$, and, on a shorter term (0.1 Myr) are $<\pm 40\%$.

^{26}Al: In most respects the situation for ^{26}Al resembles that for ^{10}Be. Its formation rate by spallation is lower, but, contrary to earlier views, the contribution of cosmic dust is apparently not significant[40]. Since any variation in the production of ^{26}Al should parallel that for ^{10}Be, and the two isotopes should have a closely similar geochemistry but different half lives (^{26}Al is 7.3×10^5 yr), it has been suggested that the decay of their ratio provides a time scale less dependent on cosmic ray fluctuations. As with ^{10}Be, ^{26}Al measurements are likely to be of direct importance mainly in the dating of sediment cores. Indirectly the information gained on cosmic ray fluctuations will help to calibrate radiocarbon back to earlier times.

The very low abundance of ^{26}Al (^{26}Al/^{27}Al$\sim 10^{-14}$ and below) and its long half life make determination by counting very difficult. ^{26}Al has been measured in ocean sediments by such means[41], and its measurement still poses a challenge to high-energy mass-spectrometric methods. A major problem is the interference from the isobar present in great excess, ^{26}Mg. One way to avoid this is to strip the ions fully, and Raisbeck[42] has used a linear accelerator as an injector to a cyclotron to produce and separate ^{26}Al^{13+} from ^{26}Mg^{12+}. Raisbeck's samples were artificially produced ^{26}Al in the region of ^{26}Al/Al 10^{-8}–10^{-11}.

^{32}Si: ^{32}Si has a half life estimated between 250 and 700 yr. It is therefore rather short for archaeological applications, unless it can be applied in contexts beyond the reach of radiocarbon. However, ^{32}Si profiles have been measured in siliceous sediments in the ocean[43,44] (with the aim of determining the sedimentation rate), and attempts have been made to use the isotope for dating ground waters[45]. However, ^{32}Si in the rain is diluted by the abundant Si isotope as the surface water equilibrates with the local geology. The solubility of Si is determined by many locally varying factors, so that application of the method is very restricted. It is difficult to conceive of any direct archaeological interest, although, of course, an understanding of the local hydrological history of a site might prove important. A clearer application could be to authenticity measurements. ^{32}Si has not so far been reported as being detected by high-energy mass spectrometry, but is obviously suitable for such an approach.

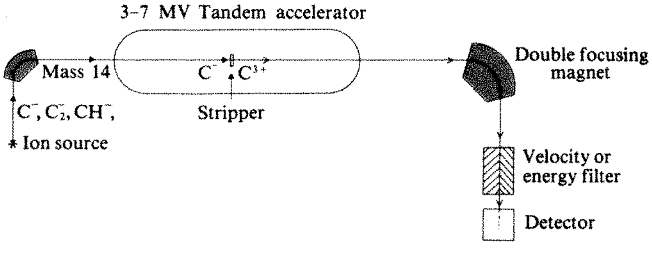

Fig. 3 High-energy mass spectrometry for the detection of ^{14}C using a tandem accelerator. Only ions of mass 14 are injected into the accelerator. Accelerated positive ions are further selected according to their magnetic rigidity and velocity or energy, before measurement of their energy loss rate and total energy in the detector.

^{36}Cl: With a half life of 0.31 Myr, ^{36}Cl would seem to have an ideal time scale for extending the archaeological dating range beyond radiocarbon. There is some resemblance to ^{14}C in that the nuclide is likely to have a fairly long residence time in the atmosphere (atmospheric sinks and sources for Cl, which although extensively studied recently, are not known quantitatively), and probably can equilibrate with the surface ocean Cl$^-$. Furthermore, a considerable proportion of ^{36}Cl may derive, not from the spallation from ^{40}Ar by cosmic rays, but from thermal neutrons by the process ^{35}Cl(n, γ)^{36}Cl. This could be important in Cl-bearing terrestrial rocks and at the ocean surface. Very few measurements of terrestrial ^{36}Cl have been made (although measurements in meteorites are relatively plentiful), and these are made more difficult by the lack of positive identification of ^{36}Cl from the weak β radiation counted. One such early study of ^{36}Cl in rain[46] concluded that considerable enhancement from bomb-testing had taken place. Note that this work required a 2,000 gallon sample. The Rochester/Toronto group[47], with a tandem accelerator, used about 1–5 l of water and achieved a background sensitivity of ^{36}C/Cl $\sim 10^{-15}$. As with ^{26}Al and ^{41}Ca, the presence of an isobar (^{36}S) in great excess is troublesome. In this case chemical methods (zone-refining) were used to reduce the ^{36}S level to a value where, although the dominant detected ion was at mass 36, the detector was no longer overwhelmed by the ^{36}S count rate.

Two types of application seem possible for ^{36}Cl (ref. 48). The concentration of ^{36}Cl in terrestrial rocks remains to be positively determined, but since 1—2 m of rock would be a sufficient shield to prevent ^{36}Cl build-up it might prove possible to date the time of exposure of a new face, or possibly the burial of formerly exposed material. For example, events such as the emplacement of a glacial erratic, burial by volcanic action, or the occurrence of earthquakes could be studied. This would be a significant extension to the range of terrestrial dating methods for the Pleistocene period. The second type of application is concerned with the solution geochemistry of Cl. The environmental measurements made so far have all concentrated on this aspect, but for quantitative work the major question is to determine the extent to which the groundwater system is closed. Again, as with ^{32}Si, an understanding of the former hydrological status of a region may be vital information in reconstructing its ecology. As for dating associated material, contemporary sediments may possibly incorporate sufficient Cl for dating purposes. Thus argillaceous sediments, possibly speleothems, as well as evaporites might be useful.

^{41}Ca: The isotope ^{41}Ca, with a half life of 0.13 Myr, is more probably formed by ^{40}Ca(n, γ)^{41}Ca than as spallation products in the upper atmosphere. Thus in many ways it resembles ^{36}Cl, and it has been suggested that it could be useful for dating in the same way[49]. Ca participates much more actively in geochemical cycles, and especially as a major constituent of bone, and so would seem to be richer in possibilities.

The equilibrium ratio ^{41}Ca/^{40}Ca at the Earth's surface would be expected to be in the region of 10^{-14}, which should not be beyond the range of high-energy mass spectrometry. However, the presence of ^{41}K as an isobar is an obvious problem. It could be possibly differentiated from ^{41}Ca by total stripping, as with ^{26}Al, but the energy and therefore the accelerator to accomplish this would have to be very large (>300 MeV), although not beyond present-day possibility.

As with ^{36}Cl, ^{41}Ca could be used to date the exposure of formerly buried rock. (The shielding depth is estimated to be about a metre.) Alternatively, the date of burial of surface material whose ^{41}Ca was in equilibrium could be studied. Since Ca is relatively abundant, its range of applications could be quite large. Again as with Cl, the dating of material formed from Ca-bearing solutions can also be attempted. The usefulness of this method will depend on how reliably the ^{41}Ca content of the relevant hydrological system can be estimated; for example, the expected level of ^{41}Ca deposited in growing bone. If a bone of known initial ^{41}Ca was rapidly buried, or left in a cave, the subsequent decay of ^{41}Ca (provided there was no further exchange) would enable it to be dated just like radiocarbon. The same would apply to the formation of speleothems, with the advantage of a cross check. Such possibilities make ^{41}Ca one of the most interesting cosmogenic radionuclides for archaeological dating, but also technically the hardest to detect. Also, as there is no equilibration with large reservoirs, local fluctuations of ^{41}Ca concentrations may render it too inaccurate to be useful.

Heavier isotopes

Heavier isotopes are also suitable candidates for detection by high-energy mass spectrometry. Their presence on Earth is due to factors such as the infall of cosmic dust, and micro-meteorites. One application, conceivably relevant to archaeology, dated the age of a meteorite fall at 4 Myr from measurements of its ^{53}Mn and ^{36}Cl concentrations[50]. Ocean sediments and volcanic deposits are likely to be the main subjects for study for ^{53}Mn (half-life 3.7 Myr) and ^{129}I (half life 17 Myr). Other isotopes could be included[18] and when their quantitative study is possible, further and unexpected details about the history of the Earth will emerge. For example, knowledge of the overall cosmic ray flux could help with ideas on the extinction of the dinosaurs.

Fission track dating

In this method[51] the accumulation of the fission events of ^{238}U in a material is measured, in contrast to the measurement of the quantity of atoms undergoing radioactive transformations. Many materials, especially glass (for example, obsidian, man made), minerals such as zircons or micas, and rock types such as tuff, basalt or granite, reveal, after etching, microscopic tracks recording each fission event. The density of tracks within a sample increases linearly with time (for archaeological time scales) and the absolute age since the formation of the sample can be derived simply by exposure to a calibrated neutron flux.

The practical application of the method depends on the availability of suitable materials, which must contain sufficient uranium, or be of great enough age, to have accumulated a measurable density of tracks. The dating of the KBS tuff already mentioned is a good example[8]. Other examples are to be found in the review by Fleischer[52], although such geological applications are not as common as might be expected. As a rule, man-made materials are too recent or contain too little uranium for useful dating by this method, although occasional exceptions, such as a high uranium glaze, are known. A more useful application is where geological tracks in a natural material have been annealed by heat, perhaps accidentally, in a man-made fire, and the time of resetting of the 'clock' can then be measured. For example, an obsidian spearhead approximately 5,000 yr old was 'dated' in this way[53].

Thermoluminescence

The well-established use of thermoluminescent dosimeters serves to demonstrate the ability of some materials to integrate α, β and γ doses as low as those occurring in the natural radiation environment of, for example, pottery. Several components of pottery itself have been found to give sufficient thermoluminescence (TL) when integrated over archaeological times. The firing of a pot erases the accumulated geological TL, and subsequent build-up of TL proceeds approximately linearly with time during the burial of a sherd, until the TL is 'read-out' by the rapid heating of a small sample (a few milligrammes, see Fig. 4). Electrons, produced as a result of the radiation dose, remain trapped in the lattice until a level of thermal energy is reached enabling them to recombine. Light produced by recombination near a luminescent centre is detected with a photomultiplier. The sensitivity of the particular sample to radiation is separately calibrated with a laboratory source. (The application to archaeological dating is reviewed in ref. 54.)

Various difficulties beset the technique and limit the accuracy that can be expected from it. Since TL relates directly to archaeological material it is potentially of great value, and much effort has gone into reducing the complications. First, the radiation dose is hard to assess accurately; the dose from surrounding burial soil is a significant contribution, and although measurable on site, is affected by burial soil moisture; the water content of the potsherd must be considered; and the likelihood of disequilibrium in the uranium and thorium daughters present in the fired clay is another difficulty. Some materials, such as felspars, are known to lose their TL with time, although this does not seem to be true of the most commonly used mineral, quartz. However, the TL response of quartz is somewhat dependent on its thermal and chemical history. It is possible to select for different components of the α, β and γ doses (which arise from internal ^{40}K, U and Th, and daughters, as well as environmental and cosmic radiation) by using grains of differing sizes[55,56]. Zircons have been used because only the internal U content need be considered[57]. Unfortunately, there is a spatial mismatch between the zoning of U concentration and the regions of high TL sensitivity in a given grain and this makes the analysis exceedingly difficult.

However, despite these drawbacks, the TL method has provided several pottery dates to an accuracy of ±5% and many at ±10%, and is useful where radiocarbon dates cannot be obtained. For old (pre-ceramic) sites, where radiocarbon dates suffer from potential contamination by modern ^{14}C, TL has been applied to such materials as hearths[58] and burnt flint[59]. There are further prospects that the method can be developed for dating lava flows, ocean sediments, and speleothems.

Summary and prospects

Clearly the ^{14}C isotope will continue to be the mainstay of archaeological dating, and is strengthened by its new-found ability to date samples up to 10,000 times smaller than before. The direct interaction of the ^{14}C cycle with archaeological events is an advantage hardly shared by the other cosmogenic nuclides. Furthermore, for dating into the distant past where much less can be assumed about environmental conditions, errors in estimates for initial values of the radionuclide at the 'start' of the clock will be more serious than is usual from radiocarbon dating. For radiocarbon an error of a factor of 2 corresponds to one half life (5,730 yr), not a disastrous error for a time scale at 30,000–100,000 yr. However, for, say ^{41}Ca, a factor of 2 variation in initial level would produce an error of 130,000 yr; the absolute error is greater for other isotopes.

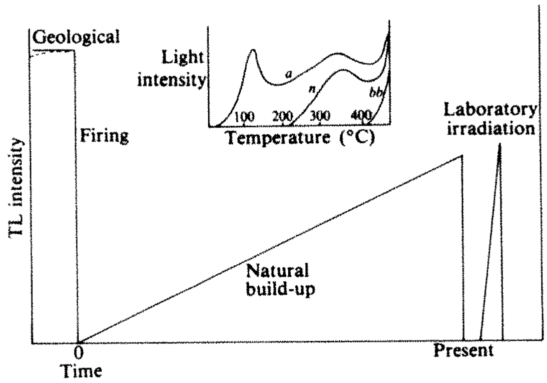

Fig. 4 The build-up of the thermoluminescent signal in pottery with time. Firing the clay erases the geological TL. In the laboratory the accumulated natural TL is measured, and then the TL from an artificial irradiation. Inset shows Glow-curves from a sample: *a*, from artificial irradiation; *n*, from naturally acquired TL over a long period of time; *bb*, the component of light due to black body radiation.

Apart from thermoluminescence, and the possible dating of bone from ^{41}Ca or by U series disequilibrium, most of the dating methods described provide direct dating only through the dating of stratigraphically associated material. This can sometimes be directly impressed by archaeological events as demonstrated by the record of hominid footprints in the Laetolil tuff of Tanzania[60], material which has now been dated by K/Ar. Note how much archaeological interpretation particularly for the Pleistocene gains from greater knowledge of the environmental changes that have taken place. Clearly ^{10}Be, ^{26}Al, ^{36}Cl in particular can provide a great deal of such information—not necessarily by dating, but by including calibration of the variation in cosmic ray flux, mixing rates for different epochs between the various terrestrial reservoirs, calibration of geomagnetic changes, and the determination of former local hydrological patterns. Much of this is related to a clearer understanding of the climatic system. Such an ambitious and complex programme is only possible if based on a great quantity of environmental measurements, which has now become possible with the application of high-energy mass spectrometry. The next decade will bring greatly increased quantitative knowledge to the study of climate and its relationship to the development of man.

There is, nevertheless, a gap in archaeological dating beyond the time scale of radiocarbon. At this age man has left few traces; bone, worked tools, fires and disturbed levels are all that we have to 'start' the radioisotope clock.

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articles

Interstellar scintillation and compact sources in supernova remnants

J. M. Cordes & J. M. Dickey

The Five College Radio-Astronomy-Observatory, University of Massachusetts, Amherst, Massachusetts 01003

Interstellar scintillation was detected from a source in or near the supernova remnant G78.1+1.8, implying an angular size $\leqslant 10^{-6}$ arc s, a brightness temperature $>10^{18}$ K, and a distance of ~3 kpc. The source does not seem to be a pulsar and is therefore the first source other than a pulsar to show interstellar scintillation. We place limits on the scintillating flux for 15 other galactic and extragalactic objects.

ALTHOUGH it is clear that some supernovae produce pulsars, it is by no means certain that all supernovae leave behind compact stellar objects nor that all such objects are pulsars. Recently[1] a new class of objects associated with supernova remnants (SNRs) has been proposed, these are radio sources with spectra markedly different from those of pulsars. While the prime candidate of this new class is probably an extragalactic source[2], one member of the class, Circinus X-1, is a galactic object that is either a neutron star or a black hole[3]. The production by supernovae of compact sources that are not pulsars exacerbates

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Table 1 Scintillation measurements on 17 radio sources

Source	Position (1950.0) α			δ			ν (MHz)	τ (min)	T_{SYS} (K)	$\bar{S}_{ISS}$ (Jy)	Notes
							Pulsar calibrator				
PSR0329+54	03 h	29 min	11 s	54°	24′	37″	850	138	167	0.60 ± 0.2	$\Delta\nu_{ISS}\approx1.2\pm0.1$ MHz
							410	39	189	0.90 ± 0.4	$\Delta\nu_{ISS}\approx35\pm10$ kHz
							Unscintillating calibrators				
0229+34	02	29	05	34	26	55	850	22	162	≤0.12	
3C123	04	33	55	29	34	14	850	54	225	≤0.15	
3C380	18	28	13	48	42	40	850	103	174	≤0.11	
3C449	22	29	06	39	06	14	850	67	164	≤0.12	
2305+18	23	05	17	18	45	12	850	89	158	≤0.11	
3C468.1	23	48	26	64	24	06	410	34	205	≤0.28	
							Program sources				
G119.5+10.0	00	14	50	71	56	24	850	298	166	≤0.07	CTA 1
G127.1+0.5	01	25	08	62	50	59	850	196	168	≤0.10	
G132.4+2.2	02	08	08	62	09	30	850	147	167	≤0.09	HB 3
G34.6–0.5	18	54	06	01	15	00	410	19	540	≤0.50	W 44
G69.0+2.7	19	51	15	32	44	00	410	25	232	≤0.30	W 56, CTB 80
G78.1+1.8	20	20	40	40	02	30	850	54	212	0.32 ± 0.1	$\Delta\nu_{ISS}\approx1.1$ MHz
							410	16	477	≤0.34	W 66, DR 4
CL 4	20	48	47	31	16	11	850	62	165	≤0.11	
Cyg X-3	20	30	38	40	47	13	850	39	185	≤0.14	
G93.6–0.2	21	22	44	50	51	12	850	78	170	≤0.10	NRAO 662, CTB 104A
NGC7089	21	30	54	–01	03	00	410	23	175	≤0.35	Globular cluster

the already problematic pulsar birth rate, which is just consistent with the supernova occurrence rate if all supernovae produce pulsars[4,5]. To detect compact sources in SNRs in general and to investigate the recently proposed class of objects in particular, we searched for interstellar scintillations (ISS) produced by electron density fluctuations in the interstellar medium, which occur if the angular size of a source is in the range 10^{-7}–10^{-3} arc s. Only pulsars have been found to scintillate in the interstellar medium although high-sensitivity observations have been made of extragalactic objects[6–9]. Here we report upper limits on the scintillating flux of six extragalactic sources, five SNRs, two of the recently proposed compact sources, and a compact source in a globular cluster. In or near one of the SNRs, G78.1 + 1.8 (also known as W66 and DR4), a scintillating source was detected at 850 MHz which is galactic and does not seem to be a pulsar.

Interstellar scintillation

Scattering of radio waves in the interstellar medium (ISM) produces a stochastic diffraction pattern at the Earth that is convected through the line of sight, thus causing time variations of the signal. The accompanying frequency structure in the spectrum of a radio source has a characteristic frequency scale that is a strong function of the observing frequency. Adopting a power-law model for the electron-density irregularity spectrum in the ISM[8,10], we have

$$\Delta\nu_{ISS} = 8.8\ \text{MHz}\left(\frac{C_N^2}{6.67\times10^{-5}\ \text{m}^{-6.67}}\right)^{-1.2}\left(\frac{\nu}{850\ \text{MHz}}\right)^{4.4}\left(\frac{L}{1\ \text{kpc}}\right)^{-2.2} \qquad (1)$$

as the decorrelation bandwidth (the half-width at half maximum of the autocorrelation function of the radio spectrum) of the ISS at a radio frequency ν. Here C_N^2 is a scale factor for the electron-density spectrum, estimated from ISS of pulsars, and L is the path length through the Galaxy to the source. The level of scintillation can be quantified by the scintillation index, m_{ISS}, which is the r.m.s. of the radio spectrum divided by the mean, and is sensitive to the angular size of the source. It can be shown[8] that

$$m_{ISS}\approx\phi_c/\phi \quad \text{where} \quad m_{ISS}^2\ll1 \qquad (2)$$

where ϕ is the source size and ϕ_c is a critical angular diameter given by[8]

$$\phi_c = 0.5\times10^{-6}\ \text{arc s}\left(\frac{C_N^2}{6.67\times10^{-5}\ \text{m}^{-6.67}}\right)^{-0.6}\left(\frac{\nu}{850\ \text{MHz}}\right)^{1.2}\left(\frac{L}{1\ \text{kpc}}\right)^{-1.6} \qquad (3)$$

While equations (1) and (3) are based on the observed scintillation properties of pulsars, the ISM is inhomogeneous and, for given sources, the predicted decorrelation bandwidths are uncertain by an order of magnitude or more. The Vela pulsar, for example, has a decorrelation bandwidth a thousand times smaller than the predicted value, largely because of the enhanced level of turbulence in the Vela SNR[10].

Observations

Observations were made in July 1978 at 850 MHz and February 1979 at 410 MHz using the 91-m transit telescope of the National Radio Astronomy Observatory. The initial frequency of 850 MHz was chosen as a compromise between maximising the scintillation index and the flux of two of the more promising sources, G127.1 + 0.5 and CL4. Our source list (Table 1) also included a pulsar (PSR0329 + 54); six non-scintillating sources of various flux densities; six SNRs with associated compact sources or with 'filled' shells; the radio source near the centre of the globular cluster NGC7089 (ref. 11); and the variable radio source associated with Cyg X-3. An autocorrelation spectrometer was used to obtain 96-point spectra with 10 MHz and

1.25 MHz total bandwidths for each of two orthogonal linear polarisations. Approximately 10–30 spectra, each with integration time 1 min, were recorded at the source position, followed by an equivalent set of off-source spectra from the same declination and 10–30 min east of the on-source position. Normalised spectra were calculated from

$$S(\nu) = T_{\text{on}}[S_{\text{on}}(\nu) - S_{\text{off}}(\nu)]/S_{\text{off}}(\nu) \qquad (4)$$

where T_{on} is the on-source system temperature and S_{on} and S_{off} are the on- and off-source spectra.

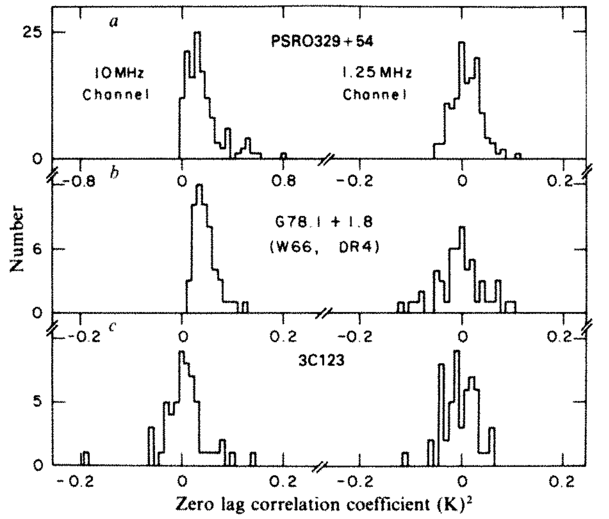

Fig. 1 Histograms of the zero-lag cross-correlation coefficient (see equation (5) with $l = 0$) for three radio sources: *a*, PSR0329+54; *b*, G78.1+1.8 (W66, DR4); *c*, 3C123. Interstellar scintillation generates frequency structure that is positively correlated between the spectra of two polarisations. Spectra with total bandwidths of 10 MHz and 1.25 MHz were analysed.

Our technique for searching for ISSs takes advantage of the fact that ISS are correlated between the two polarisations while radiometer noise and, to some extent, quasi-periodic baseline fluctuations due to standing waves are uncorrelated. Letting $S_1(k)$ and $S_2(k)$ be the sampled spectra for the two polarisations, the cross-correlation function (CCF),

$$\rho_{12}(l) = \frac{1}{N-l-1} \sum_{k=0}^{N-l-1} S_1(k)S_2(k+l),\ l = 0, 1, \ldots \qquad (5)$$

was calculated for each 1-min integration. These were summed to form an average correlation function. Histograms of the zero-lag values were formed and were extremely useful in indicating the extent to which baselines of the spectra have structure that is uncorrelated between the two polarisations. Figure 1 shows these histograms of the zero-lag points of the 1-min correlation functions for three sources. The histograms for 3C123 are symmetric about zero, as expected if radiometer noise and uncorrelated baseline fluctuations are the dominant components of the spectra. The widths of the histograms are approximately $\sigma_\rho \approx \alpha T_{\text{on}}^2/\delta\nu\tau N^{1/2}$ if only radiometer noise contributes, where τ is the integration time, N is the number of channels of bandwidth $\delta\nu$, and α is a constant of the order of unity. The histogram for the 1.25 MHz total bandwidth conforms to the radiometer noise value cf σ_ρ whereas the 10 MHz total bandwidth exceeds the radiometer noise value by a factor of 5. The excess arises from quasi-periodic baseline fluctuations with ~1 cycle every 4 MHz at 850 MHz. Such fluctuations were suppressed by subtracting a low-order least-squares polynomial from each spectrum before cross-correlating. The data presented here had a first-order polynomial subtracted. We find that the quasi-periodic baselines are generally uncorrelated between the two polarisations and that they change in amplitude and phase from one integration to the next.

ISS are evident in Fig. 1 for PSR0329+54 and G78.1+1.8 in the histograms for the 10 MHz spectra. The values of $\rho_{12}(0)$ are definitely positive for these sources, signifying that correlated structure is always present. While the presence of correlated structure is necessary to detect ISS, it is not sufficient to prove that ISS are in fact being observed. However, the shapes of the average CCFs confirm that ISS are the cause of the correlated structure. These are shown in Fig. 2. Of the 17 sources, only two, PSR0329+54 and G78.1+1.8, show correlation functions that peak at zero lag with amplitudes that are significantly larger than the magnitudes of negative excursions of the CCFs. Moreover, the shapes of the CCFs are stable from one day to the next for these sources. The quasi-periodic CCF for 3C123 peaks at a non-zero lag and attains negative values equal in magnitude to the maximum positive values as would be expected for quasi-periodic baseline structure. The CCF for the weaker calibration source 3C380 is consistent in amplitude with estimation errors expected from radiometer noise only, indicating that standing waves are not important for this source.

Note that if Faraday rotation of a linearly polarised source were significant over the total bandwidth, the resultant periodic structure would be anti-correlated between the two polarisations because the spectrum in each is proportional to $\sin^2(\beta\nu)$ and $\cos^2(\beta\nu)$, respectively, where β is related to the rotation measure. Such an effect is not apparent for any of the sources nor does the integrated linear polarisation of PSR0329+54 (which is small at 850 MHz) seem to influence the correlation functions significantly.

Table 1 shows the positions of the sources, the frequency, integration time, and system temperature; for the two sources showing ISS we list the scintillating flux density derived from

$$S_{\text{ISS}} = C[\bar{\rho}_{12}(0)]^{1/2}\, G^{-1} \qquad (6)$$

where $\bar{\rho}_{12}$ is the average correlation function, $C \approx 1.15$ is a correction factor for the flux density lost in the baseline removal process, and $G \approx 0.8\ \text{K Jy}^{-1}$ ($1\text{Jy} = 10^{-23}\ \text{erg cm}^{-2}\,\text{s}^{-1}\,\text{Hz}^{-1}$) is the system gain. A three sigma error on the scintillating flux density is

$$3\sigma_{\text{ISS}} \sim 3C\sigma_\rho^{1/2}/GM^{-1/4} \qquad (7)$$

where σ_ρ is the measured r.m.s. of the set of M zero-lag correlation coefficients for each minute of integration time. Crude estimates of the decorrelation bandwidth, $\Delta\nu_{\text{ISS}}$, were obtained by taking the lag at which the CCF crosses zero. For PSR0329+54, the two decorrelation bandwidths are consistent with values of 1.1 MHz and 43 kHz predicted by equation (1) at 850 MHz and 410 MHz, respectively,.

For the remainder of the sources, the upper limit on S_{ISS} given in Table 1 is the larger of the three sigma values given by equation (7) for the 10 MHz and 1.25 MHz total bandwidths. Although ISS were detected at 850 MHz for G78.1+1.8, they were not evident at 410 MHz above the rather high upper limit.

The scintillating source G78.1+1.8

The ISS from G78.1+1.8 cannot be easily associated with any particular source for two reasons. First, the 16 arc min telescope beamwidth at 850 MHz encompasses a complex region containing several radiosources. Second, the nature of the observations (see equation (4)) implies that ISS could have arisen from our off-source position of $\alpha(1950.0) = 20\,\text{h}\ 34\,\text{min}\ 00\,\text{s}$ and

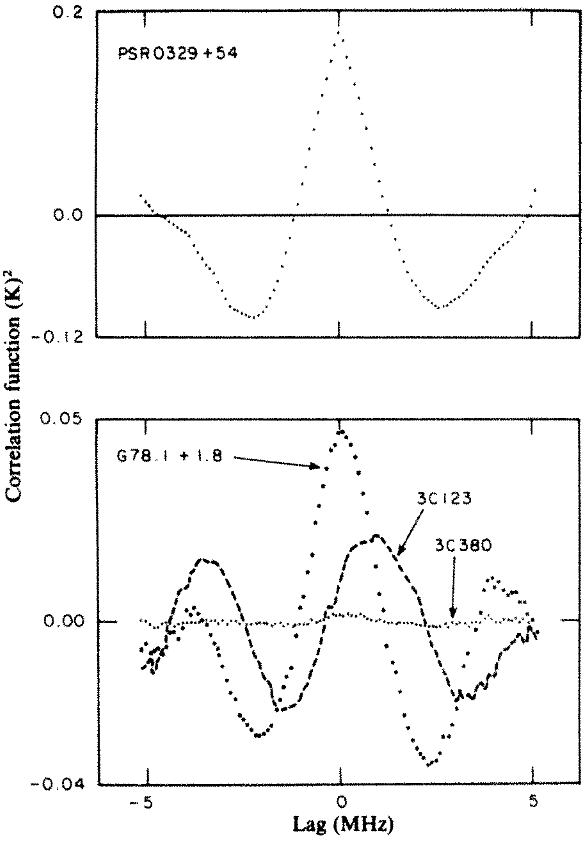

Fig. 2 Average cross-correlation functions for four sources at 850 MHz. Each lag is a multiple of the spectral resolution, 104 kHz.

$\delta(1950.0) = 40° 02' 30''$. It would not be surprising if the source were within the SNR W66, especially since the position of a γ-ray source, CG78+1, has an error box that contains W66 (ref. 12). However, the ISS may be associated with a point source[13] at $\alpha(1950.0) = 20$ h 20 min 19 s, $\delta(1950.0) = 40° 06' 09''$ which was within our beam. Although this source is variable, our observations require the flux density to have been an order of magnitude larger than the 850 MHz value expected from the measured values of 68 mJy at 1,415 MHz and 10–30 mJy at 610 MHz[13].

An obvious possibility is that the source is a pulsar. However, observations at 410 MHz using an on-line search program[14] showed no evidence of radio pulses above an average flux density of 20 mJy over a period range from 33 ms to 4 s and with a dispersion measure <120 pc cm^{-3}. The on-source and off-source positions have also been covered in two previous pulsar searches[4] that were sensitive down to at least 80 mJy. Moreover the limit on ISS at 410 MHz suggests a spectrum for the source that declines toward decreasing frequencies (that is unlike a pulsar), although the source variations may have decreased the flux density below our detection threshold during 16-min integration made at 410 MHz.

By using equations (1)–(3), we can place limits on the distance, the angular size, and the brightness temperature of the source. The similarity of the decorrelation bandwidth to that of PSR0329+54 implies a similar distance, ~2.6 kpc, although this is uncertain by at least a factor of two. Assuming this distance, we can relate the source flux density, S, to the observed scintillating flux density by

$$S = S_{\rm ISS}/m_{\rm ISS}$$

as long as $m_{\rm ISS}^2 \ll 1$. There were no unresolved sources in our beam with $S > S_{\rm max} = 1.2$ Jy[13] and therefore $\phi \leq 10^{-7} (S_{\rm max}/S_{\rm ISS})$ arc s. Consequently, the brightness temperature,

$$T_{\rm B} \equiv \lambda^2 S/2k_{\rm B}\phi^2 \geq 10^{18}\ {\rm K}$$

(where $k_{\rm B}$ is Boltzmann's constant) exceeds by six orders of magnitude the 10^{12} K maximum for incoherent electron–synchrotron radiation from a stationary source, which is limited by Compton scattering of synchrotron photons off the relativistic electrons. Although relativistic blast-wave models[15] with lorentzian factors γ can yield maximum brightness temperatures of $10^{12}\ \gamma$ K, it is probably not possible to have a source as small (linear size $\lesssim 20\%$ of a solar radius) as we have inferred without self absorption. Consequently, our results suggest that a coherent radiation mechanism is reponsible for the radiation.

We conclude that the source may be a pulsar with a period <33 ms or a pulsar of arbitrary period whose pulses are smeared out by enhanced dispersion from a region near the source. Any residual pulsation must be at a level ≤ 20 mJy or a short-period pulsar must have a flux density ≥ 0.30 mJy at 410 MHz. Either case implies an extraordinary flat spectrum as far as pulsars go. If it is not a pulsar, the source may be similar to that associated with Cir X-1, which is a binary system in which high-energy photons, produced by matter accreting on a compact object, drive a shock wave that accelerates electrons to lorentzian factors of 10^3–10^5 (ref. 3).

Compact sources in SNR

Other than G78.1+1.8, no compact sources were found in or near any of the six other supernova remnants above a flux density of ~100 mJy at 850 MHz. For G127.1+0.5 this is not surprising as the central compact source has a VLBI-determined size of 5×10^{-4} arcs s at 10.7 GHz (ref. 16). Three point sources, 4C71.01, 4C62.05, and 4C50.55, near the SNRs G119.5+10.0, G132.4+2.2, and G93.6−0.2, respectively, were not found to scintillate, signifying that such sources are indeed extragalactic or, if galactic, are too large ($\phi \gg 10^{-7}$ arc s) to scintillate at a detectable level. The SNRs with filled shells (G34.6−0.5, G69.0+2.7) do not show signs of compact sources, although our limits on these are not strong.

The point source in the globular cluster NGC7089 has a flux density of 1.3 Jy at 410 MHz and a steep spectrum ($\propto \nu^{-1.9}$) (ref. 11). Lack of ISS again argues against the source being an exotic compact object. The path length through the galaxy for this source is 1.7 kpc, assuming a disk galaxy with thickness 2 kpc, so ISS should have been detectable in the 10 MHz channel. As for Cyg X-3, the absence of ISS is not surprising because its emission seems to be consistent with an expanding incoherent synchrotron source[17].

Although our source list is too small to place meaningful constraints on the pulsar birth rate, it is clearly of interest to determine the nature of the source in or near G78.1+1.8. If it is a neutron star but not a pulsar, then the possibility exists that a significant fraction of neutron stars never manifest themselves as pulsars, therefore requiring yet more supernovae per unit time to account for the observed pulsars.

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Basalt magma sources during the opening of the North Atlantic

S. R. Carter, N. M. Evensen, P. J. Hamilton & R. K. O'Nions

Lamont–Doherty Geological Observatory of Columbia University, Palisades, New York 10964

The mantle that supplied basalts to the North Atlantic Tertiary province at the time of continental breakup was isotopically similar to the present-day sub-oceanic mantle. There is no evidence for undepleted source regions beneath the continent at this time.

THE determination of both $^{143}Nd/^{144}Nd$ and $^{87}Sr/^{86}Sr$ ratios in oceanic and continental basalts[1–5] has provided a stimulus to the evaluation of the timing and extent of differentiation in their mantle source regions. Volcanic rocks which erupted along mid-oceanic ridges comprise the largest proportion of terrestrial volcanics and may be expected to provide a good estimate of the isotopic characteristics of the sub-oceanic upper mantle. The relatively radiogenic Nd- and unradiogenic Sr-isotope compositions of mid-ocean ridge basalts (MORB) imply that the sub-oceanic upper mantle has been depleted in Rb relative to Sr and Nd relative to Sm.

The chemical character of the subcontinental upper mantle is a subject of considerable speculation. On the basis of thermal and seismic considerations, some authors[7,8] favour a markedly depleted subcontinental upper mantle. It is envisaged in these models that the subcontinental upper mantle has been depleted in basaltic constituents, producing a lower Fe/Mg ratio relative to the sub-oceanic mantle. Parts of the mantle which have lost a basaltic melt should not only have a lower Fe/Mg ratio but should also be depleted in the incompatible trace elements and have a lower Rb/Sr ratio and a higher Sm/Nd ratio than undepleted mantle. However, the Nd- and Sr-isotope compositions of continental basalts frequently indicate less depleted source regions than those supplying MORB. In some instances the $^{87}Sr/^{86}Sr$ ratios of continental basalts (which are often higher than the present-day bulk Earth value[2,4]) have been taken to infer the existence of ancient source regions beneath the continents that have time-integrated Rb/Sr ratios greater than that of the bulk Earth[9–11]. Several factors make it difficult to assess the extent to which continental basalts reflect the average properties of the subcontinental mantle. First, they typically sample only a small proportion of the mantle and are generated at unknown depths. Second, it is possible that they have been contaminated by the continental basement through which they have been erupted[12–14]. In some cases, such as the Tertiary basalts of North-west Scotland, basalts contaminated with continental crust may be readily distinguished from uncontaminated basalts by their isotope and trace element systematics. Third, if the subcontinental upper mantle is markedly heterogeneous, with strongly depleted and thus refractory regions interspersed with less depleted regions[8], melting events may be expected to sample the less depleted areas preferentially and provide a biased impression of the chemical state of the mantle.

The present project is aimed to obtain further information concerning the chemical characteristics and history of those portions of the subcontinental mantle which have undergone partial melting to produce continental basalt. To this end, Nd- and Sr-isotope compositions have been determined in plateau and flood basalts erupted between 50 and 70 Myr ago during the initial breakup and opening of the North Atlantic. The major occurrences of plateau lavas and intrusive centres associated with rifting of the North Atlantic are shown on a continental reconstruction in Fig. 1. The major period of rifting, volcanism and subsequent continental drift in the North Atlantic was heralded by a large-scale development of plateau lavas and intrusive complexes along the rifted margins of the proto-Atlantic Ocean. In North-west Scotland igneous activity began about 66 Myr ago and continued locally to about 50 Myr ago (refs 15, 16 and R. D. Beckinsale and R. J. Pankhurst, personal communication). The plateau lavas now forming the Faeroe Islands, and occuring in east Greenland and on either side of the Davis Strait in west Greenland and Baffin Island were erupted during the same period[17–19]. Of the samples analysed only those from the Faeroes are not definitely known to have been erupted through continental crust; Interpretation of geophysical data for the Faeroes area has been equivocal in this respect[20,21]. Altogether, the North Atlantic Tertiary volcanic province is comprised of some 2,000 km^2 of lavas which include mainly

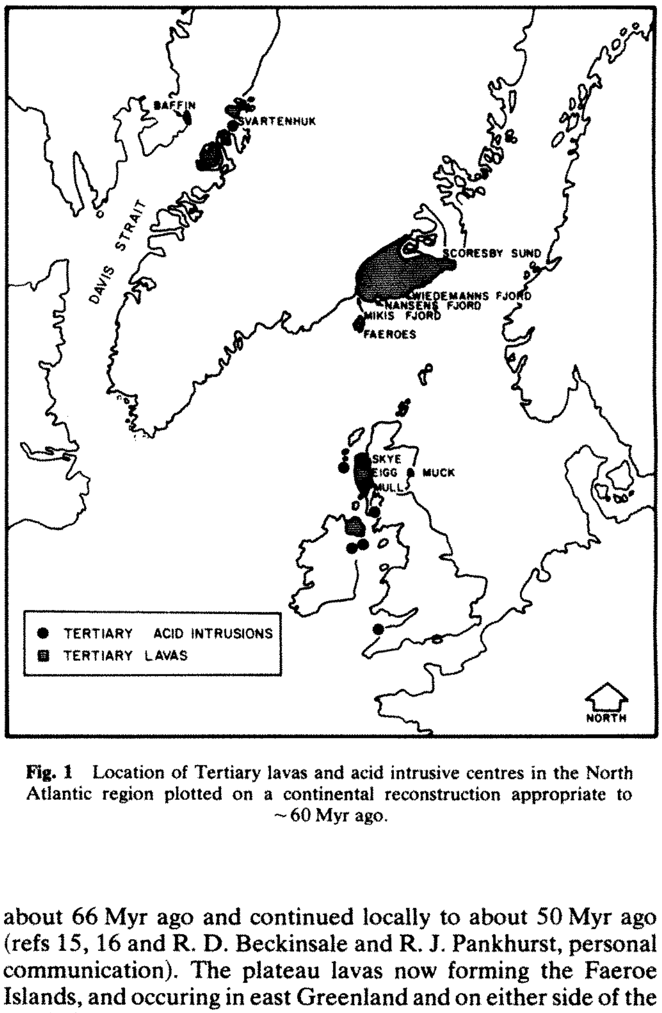

Fig. 1 Location of Tertiary lavas and acid intrusive centres in the North Atlantic region plotted on a continental reconstruction appropriate to ~60 Myr ago.

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Table 1 Rb–Sr and Sm–Nd data

Sample	Location	Rb (p.p.m.)	Rb/Sr	Sr (p.p.m.)	$^{87}Sr/^{86}Sr$	Sm (p.p.m.)	Sm/Nd	Nd (p.p.m.)	$^{143}Nd/^{144}Nd$
Faeroes									
Fo 07	Torshavn	1.19	0.004	282	0.70324±3	2.66	0.339	7.84	0.51280±2
Fo 19	Torshavn	9.02	0.07	123	0.70357±5	5.54	0.263	21.1	0.51287±3
Fo 23	Torshavn	2.53	0.009	270	0.70325±4	6.20	0.271	22.9	0.51293±2
Fo 34	Suduroy	11.1	0.041	274	0.70348±5	7.04	0.255	27.6	0.51292±2
Fo 41	Suduroy	1.87	0.005	388	0.70340±4	3.99	0.279	14.3	0.51286±3
Fo 52	Vestmanna	5.89	0.031	188	0.70337±4	4.21	0.277	15.2	0.51298±3
East Greenland									
MM20333	Mikis Fjord	10.7	0.031	349	0.70392±4	5.00	0.285	17.5	0.51288±3
CKB7147	Nansens Fjord	11.0	0.040	277	0.70361±5	6.70	0.255	26.3	0.51277±3
CKB7148	Nansens Fjord	2.53	0.006	362	0.70367±5	6.52	0.261	25.0	0.51280±2
EG 7207	Wiedemans Fjord	4.28	0.017	255	0.70363±5	5.28	0.268	19.7	0.51286±3
EG 7203	Wiedemans Fjord	13.1	0.058	244	0.70336±4	5.59	0.261	21.4	0.51283±2
EG 7126	Scoresbysund	4.35	0.020	214	0.70330±8	5.24	0.296	17.7	0.51288±2
Baffin and Svartenhuk*									
B 18	Cape Dyer	0.87	0.007	128	0.70330±4	2.24	0.299	7.50	0.51290±3
B 22	Cape Dyer	1.96	0.006	314	0.70365±4	2.01	0.294	6.83	0.51287±3
B 36	Cape Dyer	1.78	0.013	131	0.70412±4	2.73	0.320	8.53	0.51277±3
S 8	Svartenhuk	2.56	0.021	121	0.70326±4	2.74	0.302	9.07	0.51299±3
S 16	Svartenhuk	14	0.099	141	0.70302±4	2.34	0.326	7.16	0.51301±3
S 32	Svartenhuk	2.13	0.010	221	0.70374±4	5.37	0.342	15.7	0.51289±3
S 41 (trachyte)	Svartenhuk	62	0.57	108	0.70365±4	10.3	0.200	51.4	0.51287±2

$^{86}Sr/^{88}Sr$ normalised to 0.1194; $^{146}Nd/^{144}Nd$ normalised to 0.7219; ratios are initial ratios computed for 60 Myr ago. (Eimer and Amend $^{87}Sr/^{86}Sr = 0.70800$.)

* Some of the abundance data reported from R.K.O'N. and Clarke[4].

alkali olivine basalts, but also tholeiites, hawaiites mugearites and trachytes. Central intrusive complexes occur throughout the region but are most strongly concentrated in North-west Scotland (Fig. 1) and may include periodotites, gabbros and granites.

Nd- and Sr-isotopes

$^{87}Sr/^{86}Sr$ and $^{143}Nd/^{144}Nd$ ratios together with Rb, Sr, Sm and Nd concentrations have been determined in 19 basalt samples from the Faeroe Islands, east Greenland, west Greenland (Svartenhuk) and Baffin Island (Cape Dyer). Some petrological and geochemical features of basalts from these regions have been discussed previously[22–24]. The isotope ratios presented in Table 1 are computed initial ratios assuming an age of 60 Myr; small uncertainties in the estimated time of eruption result in negligible errors in the quoted initial ratios. Within each of the provinces (Faeroes, east Greenland, Baffin and Svartenhuk) there are significant variations in the initial $^{87}Sr/^{86}Sr$ and $^{143}Nd/^{144}Nd$ ratios. $^{143}Nd/^{144}Nd$ and $^{87}Sr/^{86}Sr$ exhibit a strong negative correlation in the case of the Baffin Island and Svartenhuk samples, but only a poor one in the case of east Greenland and Faeroes samples (Fig. 2). If all the samples are considered together, however, there is a good negative correlation between these two parameters, and the range of isotope compositions is similar to that for more recent volcanics erupted in parts of the North Atlantic Ocean basin (Fig. 2)[25]. More specifically, the Nd- and Sr-isotope compositions overlap with those of recent Mid-Atlantic ridge basalts and possess a similar range to Icelandic basalts erupted over the last 16 Myr (refs 4, 24). The plateau basalts are compared with the bulk Earth Rb/Sr and Sm/Nd ratios using the parameters ΔNd and ΔSr[6] in Fig. 3, from which it is evident that they were all derived from source regions previously depleted in Rb relative to Sr and Nd relative to Sm and in this respect are similar to all basalts subsequently erupted in the North Atlantic. The Rb/Sr ratios of these basalts range from 0.004 to 0.099, both less and greater than the model bulk Earth value of 0.03 (refs 2, 4), and Sm/Nd ratios range from 0.2555 to 0.342, again less and greater than the bulk Earth value of 0.31 (refs 1, 4). This feature of variable Rb/Sr and Sm/Nd ratios is not uncommon amongst basalts erupted in the ocean basins. There are no clear general correlations between the Sm/Nd, Rb/Sr, $^{143}Nd/^{144}Nd$ and $^{87}Sr/^{86}Sr$ ratios in these basalts.

Localisation of crustal contamination

In addition to the analyses presented here, Nd- and Sr-isotope data are also available for Tertiary basalts from North-west Scotland (Skye, Mull, Eigg and Muck)[14]. The major contrast between the North-west Scotalnd basalts and those from the other locations in the North Atlantic province is the clear divergence of the $^{143}Nd/^{144}Nd$ and $^{87}Sr/^{86}Sr$ ratios of some basalts from the trend defined by the results presented here and for other recent North Atlantic basalts (Fig. 4). These divergences have previously been attributed to contamination of the basalts by the 2.9-Gyr-old Lewisian basement gneisses through which they were erupted. In some instances this contamination involved Lewisian granulite facies material with negative ΔNd values in the range −30% to −40% and ΔSr values between −20% and −40%. Elsewhere, Lewisian amphibolite facies material with similar ΔNd values, but with positive ΔSr values of more than 100%, seems to have been the contaminant.

So far, there is no evidence for basalt contamination by Precambrian basement elsewhere in the Tertiary North Atlantic.

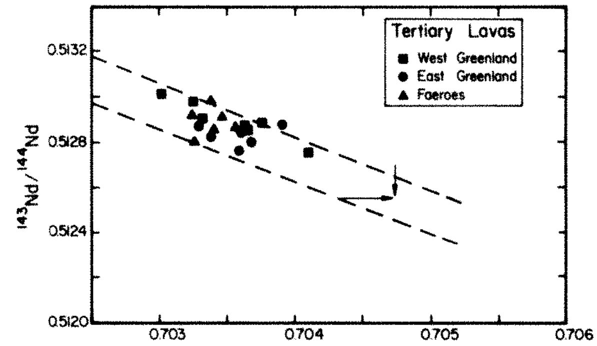

Fig. 2 Comparison of $^{143}Nd/^{144}Nd$ and $^{87}Sr^{86}Sr$ ratios in North Atlantic volcanics (Table 1) recalculated for their time of eruption 60 Myr ago and compared with the bulk Earth $^{143}Nd/^{144}Nd$ and $^{87}Sr/^{86}Sr$ ratios (indicated by arrows) and inferred bounds of contemporary Atlantic basalt source regions at that time.

It is evident that contamination is a common but localised feature of the North-west Scotland basalts and correlates with the concentration of Tertiary acid intrusive centres in this region (Fig. 1). The Pb-, Nd- and Sr-isotope compositions of the acid intrusives in Skye, North-west Scotland[14,26], indicate that Lewisian basement is a major component of their source. This may reflect overall higher temperatures in the lower crust in North-west Scotland at the time of basalt eruption than elsewhere in the North Atlantic Tertiary province.

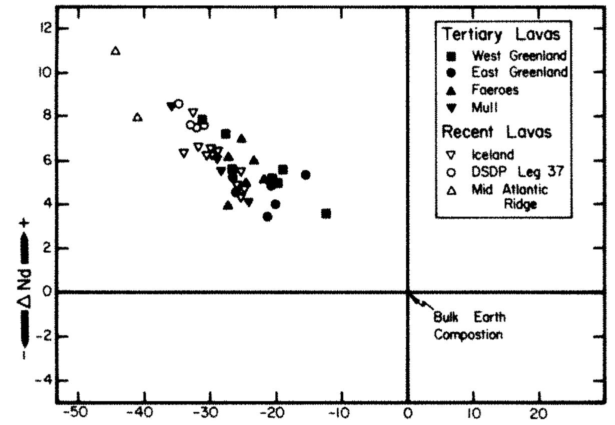

Fig. 3 Comparison of ΔNd and ΔSr values in Tertiary lavas from Baffin Island, east and west Greenland, Faeroes, Mull and Recent volcanics from the North Atlantic[4]. The solid square symbol is used for both Svartenhuk (west Greenland) and Baffin Island samples. ΔNd is the percentage deviation of the time-integrated Sm/Nd calculated from the measured $^{143}Nd/^{144}Nd$ ratio from the bulk Earth value, and ΔSr is the analogous parameter for Rb/Sr ratios[6].

Evolution of the North Atlantic source regions

The Nd- and Sr-isotope compositions of the basalts reported here and those of uncontaminated basalts from Northwest Scotland[14] all indicate derivation from source regions previously depleted in Rb relative to Sr and Nd to Sm. This observation is reflected in their uniformly positive ΔNd and negative ΔSr values. There is no evidence for the involvement of undepleted (or pristine) mantle source regions in the generation of basalts during the breakup of the North Atlantic, or indeed any basalt samples with either positive ΔSr or negative ΔNd values which are not readily explained by crustal contamination. The isotopic evolution of the subcontinental mantle sources sampled in the Tertiary seems to have been similar to that of some of the sub-oceanic sources sampled subsequently during the generation of the North Atlantic Ocean basin and particularly those represented by Icelandic volcanics erupted during more recent times (Fig. 3).

It is interesting to compare these conclusions with inferences concerning the chemical characteristics of subcontinental mantle based on geophysical observation and other continental volcanic suites. Both Jordan[7] and Oxburgh and Parmentier[8] have suggested that the subcontinental mantle must be overall more depleted in basaltic constituents than suboceanic mantle in order to stabilise it against convective disruption. If the degree of depletion of subcontinental mantle were uniform and had occurred over the same time scale as the depletion of present-day sub-oceanic mantle, continental basalts would have extreme values of ΔNd and ΔSr, similar to those of MORB. However, the isotopic characteristics of Tertiary North Atlantic basalts are more similar to those of some contemporary Icelandic basalts. If the Tertiary basalts were derived from the less depleted parts of the subcontinental mantle and other more-depleted and refractory areas did not melt, the basalts may convey a biased impression of the degree of depletion. Alternatively, and perhaps more likely, any depleted subcontinental mantle existing beneath the region before breakup may have been disrupted and replaced by less-depleted mantle material at a higher temperature and with a geological history similar to that of mantle material subsequently sampled during the evolution of the North Atlantic Ocean basin.

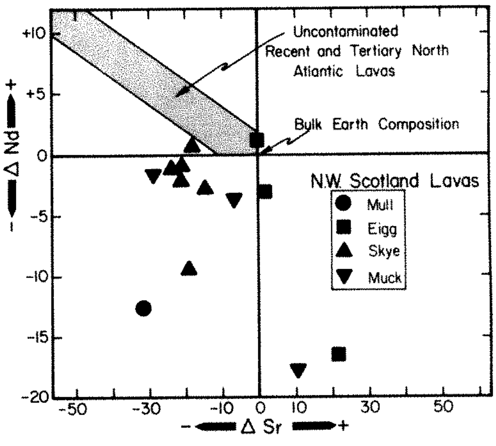

Fig. 4 Comparison of ΔNd and ΔSr parameters for Tertiary and Recent uncontaminated volcanics from the North Atlantic with Tertiary North-west Scotland volcanics inferred to have been contaminated by Lewisian basement[5]. ●, Mull; ■, Eigg; ▲, Skye; ▼, Muck.

Although the data obtained for the Tertiary North Atlantic basalts do not refute claims for the existence of undepleted or enriched subcontinental mantle source regions[2,9], they do clearly demonstrate that such potentially fertile regions were not available to produce basalts beneath the North Atlantic region at the time of incipient breakup in the early Tertiary.

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A role for brown adipose tissue in diet-induced thermogenesis

Nancy J. Rothwell & Michael J. Stock

Department of Physiology, Queen Elizabeth College, University of London, London W8, UK*

Measurement of energy balance during voluntary overeating in rats unequivocally establishes the quantitative importance of diet-induced thermogenesis in energy balance. Like cold-induced thermogenesis, this form of heat production involves changes in the activity of the sympathetic nervous system and brown adipose tissue which suggest that this tissue may determine metabolic efficiency and resistance to obesity.

IT is generally accepted that heat production in mammals varies, but there is considerable controversy about the factors which cause this variation. Cold-adapted animals show non-shivering thermogenesis (NST) in which increased heat production, not associated with muscular activity, is mediated by the sympathetic nervous system and the animals show a pronounced increase in sensitivity to the thermogenic and lipolytic effects of catecholamines[1,2]. The capacity for NST is related to the presence of brown adipose tissue (BAT) which is found in many cold-adapted animals, hibernators and the neonates of many species, including man[3]. *In vivo* measurements indicate that, in spite of its small total mass (1–2% of body weight in the cold-adapted rat), under the influence of catecholamines BAT can receive as much as 34% of cardiac output and probably accounts for up to 60% of NST in the cold-adapted rat[4]. The significance of diet-induced thermogenesis (DIT) remains controversial. It is generally agreed that genetic obesity in mice is, in part at least, due to a failure of DIT[5,6] (associated also with a failure in NST[6,7]). There is also considerable evidence to show that variation in the energy cost of weight maintenance or weight gain can be induced in rats[8–11], pigs [8,12] and man [13–15] by manipulating dietary composition or intake. Miller [16] has argued that DIT has a central role in the regulation of energy balance, whereas Blaxter[17] has suggested that DIT is relatively unimportant. The controversy arises mainly from the use of abnormal animals and diets to produce differences in energy intake, in contrast to the voluntary overfeeding used in human studies. We have now developed a new dietary regimen that induces voluntary overeating of a balanced diet in normal animals and unequivocally demonstrates the quantitative importance of DIT in energy metabolism. As with NST, there is a sympathetic/catecholamine involvement and changes in the size and metabolism of BAT suggest that this tissue is an effector of DIT as well as NST.

'Cafeteria' feeding

We have used a dietary regimen originally described by Sclafani and Springer[18] which we call 'cafeteria' diet. Rats are offered various palatable food items in addition to normal laboratory stock diet. Each day four new food items are offered and the rat, which normally controls its intake precisely, is induced to overeat. On this diet animals rapidly become obese but when palatable food items are removed, and only stock diet is available, body weight rapidly returns to the level of animals fed stock diet throughout[19]. This reversible obesity seems to have no lasting effects on metabolism: three repetitive bouts of treatment do not affect subsequent ability to lose weight[20].

The degree of obesity obtained using the cafeteria diet varies and initially this was ascribed to a failure in some experiments to induce hyperphagia. However in all experiments where food intake was measured there was evidence for marked hyperphagia, suggesting that differences in weight gain were due to variations in heat production.

Adult male Sprague-Dawley rats (from Charles River) were allowed free access to water and a pelleted stock diet. Half of the animals were maintained on the cafeteria diet for 21 days after which all animals continued on stock diet only. Detailed measurements of energy balance were made on six animals from each group and the results are shown in Table 1. In spite of consuming 80% more energy, the weight gain of cafeteria-fed rats was only 27% greater than that of controls. The hyperphagia was due to a combination of a greater weight of food eaten per day and the higher energy density of the cafeteria foods, and was still apparent when energy intakes were corrected for differences in body size.

Analysis of body composition revealed that 93% of the excess weight gain of cafeteria rats consisted of fat deposition, resulting in a large increase in body energy content (Table 1). However, the energy cost of weight gain (g gain per MJ eaten) was significantly lower in cafeteria rats (11.23±0.55) than

Table 1 Energy balance data for control and cafeteria rats from day 0 to day 21

	Control	Cafeteria
Body weight gain (g)	103±7	131±8*
Body fat gain (g)	40±3	66±5†
Energy intake (kJ)	6,480±200	11,670±330‡
($kJ/W^{0.75}/day$)	650±10	1,140±30‡
Final body energy (kJ)	4,100±160	4,540±120*
Body energy gain (kJ) (days 0–21)	1,790±160	2,230±130*
Efficiency of gain (kJ gain per kJ eaten)	0.36±0.07	0.22±0.01†
Energy expenditure (kJ)	4,690±100	9,440±250‡
($kJ/W^{0.75}/day$)	470±10	930±20‡

Initial body weight (g) on day 0 was; control 318±4, cafeteria 315±4. Energy intake was estimated from (1) weight of stock diet (PRD, Christopher Hill Group Ltd) consumed and its metabolisable energy density (10.75 kJ per g, previously determined experimentally) and (2) the weight of each cafeteria food item consumed and the metabolisable energy density given in food composition tables[21]. These animals were killed on day 21, body composition determined by analysis and body energy calculated from this using values of 38.0 and 19.16 kJ per g for the energy value of fat and protein respectively[22]. A group of six animals were also killed on day 0, body energy content determined in the same way and this value subtracted from the final body energy content of the experimental animals in order to estimate body energy gain. The initial body composition of this baseline group and the experimental animals was also determined indirectly on day 0 using an *in vivo* tritium dilution method[23]. The composition of both experimental groups was identical and no different from that of baseline animals. Energy expenditure over the 21 days of the experiment was estimated by subtracting the body energy gain from the metabolisable energy intake $W^{0.75}$ = body weight $(kg)^{0.75}$. Mean values ±s.e.m., $n=6$, * $P<0.05$, † $P<0.01$, ‡ $P<0.001$ compared to controls.

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* Permanent address: Department of Physiology, St George's Hospital Medical School, London SW17, UK.

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controls (15.89 ± 0.67, $P < 0.01$) and body energy gain per unit intake was similarly reduced by 39% in experimental animals (Table 1). This contrasts with our report[24] that efficiency (g gained per MJ) was not significantly increased in cafeteria rats and may be due to inherent differences in metabolic efficiency between diverse groups of experimental animals. In a recent experiment with the same strain of rat from two different breeding colonies (Tuck, Essex and Charles River, Kent) the efficiency of weight gain of 'Tuck' rats fed the cafeteria diet was 12% greater than the stock fed controls, although not significantly so, whereas the efficiency of 'Charles River' cafeteria animals was 17% lower than their controls ($P < 0.01$). The genetic influence on metabolic efficiency is well known[25,26], but the above results illustrate that even within the same strain of rat, small differences in genetic and/or environmental background can exert a profound influence on the metabolic response to overfeeding.

Our studies show some parallel with those carried out in man, particularly the overfeeding experiments[13-15]. In human subjects, changes in the energy cost of weight gain have been ascribed to increases in DIT, and our energy balance results confirm a similar effect in the overfed rat. There was a 100% increase in the total energy expenditure of cafeteria-fed rats, a difference which is still apparent when the results are corrected for the greater body weight of these animals (Table 1). It is unlikely that such large differences in energy expenditure could be accounted for by activity which, at best, represents only a small fraction of energy expenditure in the rat and these adult rats were housed in pairs in cages designed to restrict activity. The differences in daily heat production observed would require prodigious activity (equivalent to each rat walking 6 km per day). It is also unlikely that these differences were due to the energy cost of the excess fat gain in cafeteria rats. Less than 8% of the extra energy expended by cafeteria rats could be accounted for in this way if a value of 14 kJ per g (ref. 27.) is assumed for the energy cost of fat gain.

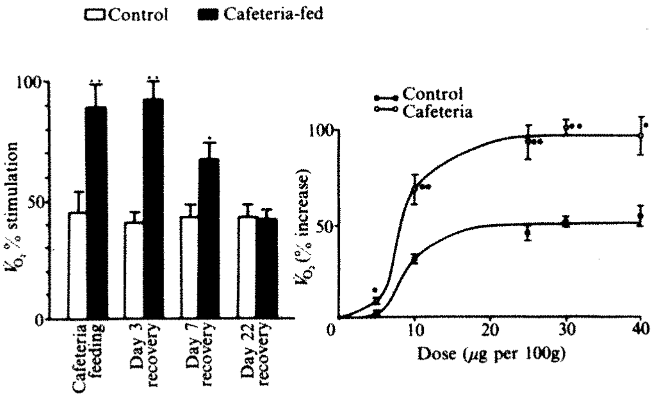

Fig. 1 *a*, Effect of noradrenaline on oxygen consumption in cafeteria and control animals. Adult, female Sprague-Dawley rats were fed the cafeteria diet for 28 days and noradrenaline sensitivity was measured on day 26. The cafeteria diet was withdrawn on day 28, allowing free access to stock diet and noradrenaline sensitivity was measured 3, 7 and 22 days later. Cafeteria rats were 50 g heavier than controls on day 26 and had completely returned to control weight by day 22 of recovery. Oxygen consumption was measured for 2 h before and after injection of noradrenaline (Levophed, 25 μg per 100 g body weight, s.c.). Results are presented as oxygen consumption after noradrenaline as a percentage of the pre-injection value. Mean values, bar denotes s.e.m. * $P < 0.05$, ** $P < 0.01$, compared to controls. *b*, Stimulation of resting oxygen consumption by various doses of noradrenaline. Adult female Sprague-Dawley rats were maintained on the cafeteria diet for 15 days and during this time gained 23 g excess weight compared to their free-feeding controls. Two days after withdrawal of the mixed diet resting oxygen consumption was determined for 2 h before and after administration of noradrenaline. One dose of noradrenaline was tested in four control and four experimental animals and the withdrawal of the cafeteria diet was staggered in order to facilitate these measurements. Mean values, bar denotes s.e.m., $n = 4$. * $P < 0.05$, ** $P < 0.01$, compared to controls.

Table 2 Resting oxygen consumption (ml/min/$W^{0.75}$) of control and experimental rats in response to propranolol, during and after cafeteria feeding

	Control	Cafeteria
Cafeteria feeding period		
Day 5	11.91 ± 0.41	14.48 ± 0.16†
Day 11	11.74 ± 0.25	14.11 ± 0.46‡
Day 13	10.82 ± 0.28	14.05 ± 0.45†
Day 16	10.65 ± 0.24	13.65 ± 0.58†
Propranolol injected (day 13)		
Before	10.82 ± 0.28	14.05 ± 0.45
After	10.85 ± 0.29	11.80 ± 0.26
	NS	++
Recovery period		
Day 1	10.73 ± 0.16	12.21 ± 0.38†
Day 2	10.14 ± 0.14	12.30 ± 0.23†
Day 3	10.32 ± 0.17	11.49 ± 0.41*
Day 13	10.61 ± 0.28	10.50 ± 0.29(NS)
Propranolol injected (day 2)		
Before	10.14 ± 0.14	12.30 ± 0.23
After	10.15 ± 0.19	10.88 ± 0.27
	NS	++

Resting oxygen consumption was measured in a closed circuit respirometer[28] for 2 h during the day, at a temperature of 29 ± 1 °C. On day 13 of cafeteria feeding and day 2 of the recovery period oxygen consumption was measured before and after injection of propranolol (0.5 mg per 100 g body weight s.c.). Resting values were obtained as described in ref. 29 and have been corrected for differences in body size. The same animals were used for these measurements throughout the 21 day cafeteria feeding period and the withdrawal of the mixed foods. Oxygen consumption was determined on the first 3 days of the recovery period since it is during this time that weight loss is most rapid and the measurement on day 13 was made when the body weight of the cafeteria rats had returned to control levels. Mean values ± s.e.m., $n = 6$: * $P < 0.05$, † $P < 0.01$, ‡ $P < 0.001$, compared to controls: ++ $P < 0.01$ compared to pre-injection value, NS not significant.

The association of lowered efficiency with increased DIT in the overfed animals was confirmed by measuring resting oxygen consumption ($\dot{V}_{O_2}$ (Table 2). Because these represent sample daytime measurements in resting animals they exclude the effects of activity and night-time feeding. Nevertheless, a 20–30% difference in $\dot{V}_{O_2}$ was consistently observed between control and experimental rats throughout the period of weight gain and, confirming our previous findings[19,20,24], also during the initial recovery period when cafeteria rats were losing weight rapidly. This sustained increase in DIT after the cafeteria diet was withdrawn is one of the most interesting effects of this treatment since during the recovery period animals are hypophagic[19,24]. Thermogenesis was then dependent on the degree of obesity and $\dot{V}_{O_2}$ returned to control levels as body weight declined to normal. This independence of DIT from the immediate energy intake indicates a chronic metabolic adaptation to hyperphagia and obesity.

Unlike previous demonstrations of DIT, our results clearly establish the cafeteria system as a method for inducing DIT in normal rats without resorting to nutritionally-unbalanced diets (such as low-protein diets) and we can now consider the metabolic origins of DIT using this model.

Metabolic changes

Increased sensitivity to the thermogenic effects of noradrenaline in cafeteria-fed rats[20] is illustrated in Fig. 1. In experimental animals the increase in $\dot{V}_{O_2}$ after noradrenaline was twice that seen in controls, both during the period of weight gain and when cafeteria feeding was stopped, after 3 days of weight loss: these differences diminished as the animals returned to control body weight. In those rats described in Table 1 there was also a greater sensitivity to noradrenaline during the period of weight gain with the control and cafeteria rats showing increases in $\dot{V}_{O_2}$ of 6% and 76% respectively. To confirm that differences in sensitivity to noradrenaline were not a reflection of the dose used, doses of 5–40 μg per 100 g body weight were tested (Fig. 1*b*). Cafeteria animals were more sensitive throughout the range: response was maximal for approximately the same dose given to both groups (25 μg per 100 g), whereas the lowest dose (5 μg per

100 g) produced a significant response in cafeteria rats only. There was no correlation between noradrenaline sensitivity and body fat content in either group ($r < 0.3$) indicating that the enhanced sensitivity was not simply a consequence of a greater fat mass.

There are many reports of enhanced sensitivity to the thermogenic effects of noradrenaline in animals exhibiting NST and the differences between warm and cold-adapted rats[30,31] are as great as those seen in control and cafeteria animals. The effects of increased sympathetic activity on oxygen consumption in cold adapted rats can be inhibited using agents that block catecholamine receptors[32] and might therefore be expected to have similar effects on DIT. Table 2 shows that the β-adrenergic blocking agent propranolol produced a significant decrease in the $\dot{V}_{O_2}$ of cafeteria rats while on the mixed diet and during the early recovery phase, but had no effect on the oxygen consumption of control animals. The changes in noradrenaline sensitivity and the effects of propranolol on $\dot{V}_{O_2}$ therefore indicate an increase in sympathetic activity in cafeteria-fed animals and suggest that the sympathetic nervous system is involved in the control of DIT as well as NST. Further support for this idea comes from the report by Young and Landsberg[33] of comparable increases in cardiac noradrenaline turnover between overfed rats and rats adapted to the cold.

Such results and the similarities between NST and DIT inevitably lead to the question of whether the same effector tissue is involved in the two types of thermogenesis. As stated above, the main effector tissue for NST is BAT. It has not previously been suggested that BAT plays a thermogenic role in any situation other than cold exposure. However, we have found increased deposits of BAT in the interscapular region of cafeteria rats and this would be consistent with other similarities we have noted between these rats and cold-adapted animals.

Because BAT is highly thermogenic it can be detected, particularly in the interscapular region, using the non-invasive technique of measuring surface temperature[34]. Surface and rectal temperatures were therefore measured in control and cafeteria rats before and after injection of noradrenaline 25 μg per 100 g (Fig. 2). Skin temperature was measured between the scapulae where large deposits of BAT are seen in cold-adapted rats and on the abdomen directly over the liver. Before injection of noradrenaline mean rectal and interscapular temperatures of cafeteria rats were, respectively, 0.6 and 0.8 °C higher than those of controls ($P < 0.001$) but abdominal temperature was similar in the two groups. Noradrenaline produced a marked rise in skin and rectal temperatures of experimental animals but did not significantly alter those of controls. The interscapular temperature increased by 1.3 °C after noradrenaline compared to only 0.7 °C for the abdomen, so that the interscapular temperature of cafeteria rats was significantly higher than abdominal after noradrenaline ($P < 0.001$). This marked increase in skin temperature over the interscapular BAT in cafeteria rats after noradrenaline indicates either an increase in heat production of this tissue and/or an elevated blood flow, both of which are seen in the cold-adapted rat after noradrenaline[4].

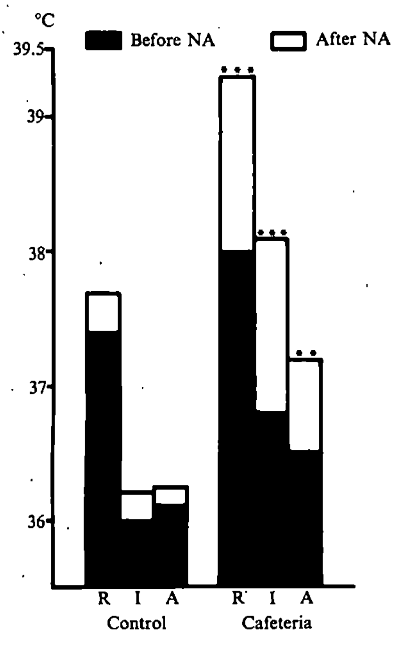

Fig. 2 Rectal (R), interscapular (I) and abdominal (A) temperatures of control and cafeteria rats before and after noradrenaline. Rectal and skin temperatures were measured on day 19 of cafeteria-feeding using thermocouples sensitive to 0.05 °C (Comark 1694 F) in an air-conditioned room at 24 ± 1 °C. Skin temperature was determined between the scapulae and on the abdomen just below the xiphisternum by taping a thermocouple over the surface of the skin and rectal temperature was measured by insertion of a plastic coated thermocouple 4 cm into the animal. Three readings were taken at 5 min intervals for each site before injection of noradrenaline (25 μg per 100 g body weight) and a further three measurements at 5-min intervals were carried out 1.5 h after administration of the drug. Mean values, $n = 6$. ** $P < 0.01$, *** $P < 0.001$, compared to pre-injection temperature.

Table 3 Brown adipose tissue weight, composition and *in vitro* release of free fatty acids (FFA)

	Control	Cafeteria
Interscapular BAT weight		
(mg)	266 ± 13	692 ± 28‡
(mg per 100 g body wt)	63 ± 3	156 ± 9‡
Interscapular BAT composition		
% Fat	36.3 ± 1.9	36.8 ± 2.3
% Fat-free dry wt	8.7 ± 0.8	9.8 ± 1.1
In vitro FFA release (μM FFA per mg per 2 h)		
Basal	5.54 ± 0.17	5.09 ± 0.29
Noradrenaline stimulated	7.33 ± 0.35	11.70 ± 0.59‡
% Increase	33 ± 7	132 ± 15‡

The fat content of brown adipose tissue was determined by ether extraction. Basal *in vitro* release of FFA was estimated using duplicate 50 mg samples. Each sample was incubated in 1 ml Earle's bicarbonate (pH 7.4, containing 2% bovine serum albumin), at 37 °C in a shaking water bath for 2 h. Stimulated FFA release was measured in another set of duplicates with noradrenaline (final concentration 2.5 μg ml^{-1}) added to the medium. After incubation the tissue was removed and the FFA content of the medium was determined by a micro-colorimetric method[35]. Mean values ± s.e.m., $n = 6$, ‡ $P < 0.001$ compared to controls.

These animals were killed after 21 days of cafeteria feeding and interscapular BAT was dissected out. The weight of tissue found in cafeteria rats was similar to that found in rats adapted to 5 °C[30,36] but was more than twice that found in controls (Table 3). The observed increase was due to an increase in active tissue mass rather than excessive lipid deposition (Table 3) suggesting a true hypertrophy in response to cafeteria feeding. Table 3 also shows that although basal release of free fatty acids (FFA) from interscapular BAT was similar for both groups, the stimulated release in the presence of noradrenaline (% increase) was four times greater in cafeteria rats.

Thus by various criteria (interscapular BAT mass, temperature response and lipolytic sensitivity to noradrenaline) it is possible to demonstrate an association between changes in BAT and DIT that resemble the changes seen in cold-adapted animals. The contribution of BAT thermogenesis to total heat production has yet to be quantified but there is a remarkable correlation between resting $\dot{V}_{O_2}$ of cafeteria rats and their interscapular BAT mass ($r = 0.8$, $n = 12$, $P < 0.001$) which is not apparent in control animals ($r = 0.45$, $n = 12$, not significant). If such a relationship were causal it would not necessarily exclude the participation of other tissues in the metabolic response to overfeeding and we have shown that there are increases in the lipolytic sensitivity of white adipose tissue to noradrenaline in cafeteria rats[20]. Nor can other thermogenic hormones, in particular the thyroid hormones be excluded. The role of the thyroid in NST is unclear (see ref. 1) but injections of thyroid hormones have been shown to induce hypertrophy of BAT, increase noradrenaline sensitivity and improve cold tolerance[30]. In addition, recent studies have demonstrated that circulating levels of triiodothyronine (T_3) are elevated in overfed human subjects[37] and in animals exhibiting DIT in response to low-protein diets[11,12]. It is therefore not surprising that plasma T_3 levels were elevated by 27% in cafeteria rats during mixed diet feeding (T_3 (ng ml^{-1}): control 0.89 ± 0.03, cafeteria 1.14 ± 0.08, $P < 0.01$). This difference was still apparent 4 days after the mixed diet was withdrawn when cafeteria rats were in fact hypophagic compared to controls but still had a higher oxygen consumption. These higher T_3 levels may not necessarily be directly responsible for the elevated heat production of cafeteria rats but could explain the hypertrophy of BAT and enhanced sensitivity to noradrenaline seen in these animals.

Brown fat in man

We have pointed out[38] that obesity induced by cafeteria feeding has several advantages over other experimental models of obesity (such as genetic, hypothalamic and drug-induced obesity). Apart from the fact that cafeteria feeding does not involve pathological and irreversible changes, it is perhaps the closest approximation to the human condition where, at least in some parts of the world, a varied and highly palatable diet encourages over-indulgence. In spite of this, many individuals remain lean, perhaps because they, like the rat, exhibit DIT. One major difference between the rat and man is that although adult man does possess some BAT[39,40] this was not considered to be thermogenically active. However, the association between DIT and BAT in the rat demands a reconsideration of the role of this tissue in all species, including man, and promoted us to undertake the experiment shown in Table 4. It was postulated that the existence of functional BAT in man could be identified by local changes in skin temperature during stimulation by a sympathomimetic agent such as ephedrine, which increases metabolic rate in man[41]. Ephedrine produces the largest increases in skin temperature in the neck and upper back—areas that correspond to BAT locations in the human neonate and adult[34,39]. The existence of these localised areas which are sensitive to ephedrine has also been confirmed using IR thermography (Fig. 3). These findings can be interpreted as evidence for functional BAT in man.

Conclusions

By adopting the cafeteria feeding system we have been able to demonstrate the existence and importance of DIT in energy metabolism. The striking similarities between DIT and NST suggest that these two phenomena may have a common metabolic origin in BAT. The importance of NST is mainly restricted to cold-adaption in small mammals. The role of BAT in man is not clear, but our findings now raise questions as to the advantages of possessing this energetically wasteful tissue. Confirmation of the thermogenic activity and quantitative significance of BAT in man must await more direct methods for studying local tissue heat production and blood flow but if BAT in adult man has an oxidative capacity similar to that of other species it could make a significant contribution to energy expenditure. Similarly, a defect in, or an absence of BAT would predispose to obesity. The finding[42] of a decreased thermogenic response to noradrenaline in constitutionally obese humans is similar to that reported in the genetically obese mouse[6]. If, as in the obese mouse, this loss of sensitivity is associated with a defect in BAT[43] the importance of this tissue in man will assume clinical significance.

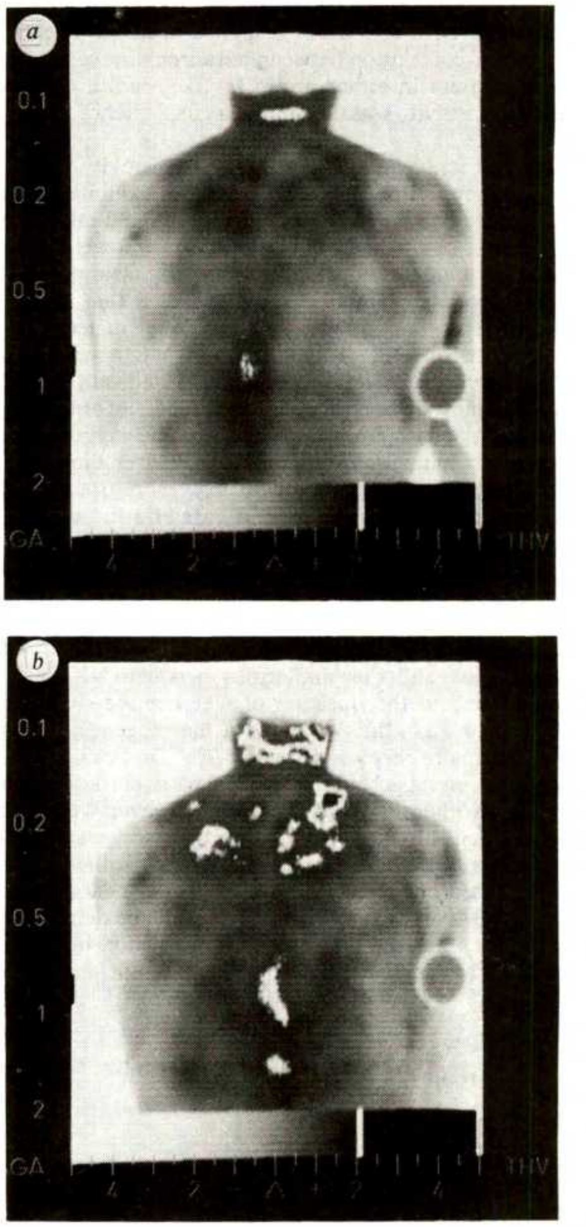

Fig. 3 IR thermograms of one subject before and after administration of ephedrine. IR thermograms (AGA Thermovision; 2–6 μm) were made after the subject had equilibrated for 12 min at a room temperature of 19 °C. Before taking ephedrine (*a*) the highest skin temperature recorded, which corresponds to the vertical bar on the lower scale, was 34.1 °C. The areas of skin isothermal to this temperature appear as white patches on the image. One hour after taking ephedrine (*b*) the areas at this temperature have increased but are concentrated between and above the scapulae and in the neck region. The subject was male, aged 36 yr and the dose of ephedrine was the same as in Table 4.

Table 4 Changes in skin temperature (Δ °C) following the oral administration of ephedrine

	Subject A	Subject B	BAT site
Nape of neck	0.4	0.8	+
Interscapular	0.7	0.6	+
Suprascapular	0.4	0.4	NE
Subscapular	0	0.5	NE
Lower spine	0.1	−0.1	NE
Abdomen	−0.1	−0.1	−
Sternum	0.4	0.7	+
Shoulder	−0.3	0.2	NE
Oral	0.1	−0.2	

Skin temperatures were recorded on the unclothed torso before and after taking ephedrine (1 mg per kg body weight), using a multi-channel electronic thermometer (Comark 1604). The skin thermocouples were kept in place for the duration of the experiment which was conducted in a room maintained at 25 °C. Subject A was male, aged 36 yr and subject B was female, aged 23 yr. The symbols in the column headed 'BAT site' refer to BAT locations in adult man as described by Heaton[39] (+, positive identification; −, negative; NE, not examined).

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Expression of the chromosomal mouse β^{maj}-globin gene cloned in SV40

Dean H. Hamer & Philip Leder

Laboratory of Molecular Genetics, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20205

The complete chromosomal mouse β^{maj}-globin gene, including its intervening and flanking sequences, has been cloned in the monkey virus SV40. The mouse gene is transcribed, processed and translated in infected monkey kidney cells to yield mouse β^{maj}-globin.

WE have identified several conserved sequences in the chromosomal DNA of cloned mouse α- and β-globin genes that may have an important role in their ordered expression and in the processing of their initial transcripts[1-5]. Despite the fact that these sequences have been preserved during several hundred million years of separate evolutionary development, we cannot be certain of their function or importance until they, or mutants derived from them, have been tested in an appropriate biological system. Our first approach to the development of such tests has involved the use of animal viruses as vectors of chromosomal genetic information. Here, we have inserted the complete genomic mouse β^{maj}-globin gene into the monkey virus SV40 and used the resulting recombinant to infect cultured monkey kidney cells. Despite the species and cell-type differences, the mouse globin signals for RNA splicing, polyadenylation and translation are recognised in this host, and substantial quantities of mouse β^{maj}-globin are produced. Thus, the SV40–monkey cell system seems to provide a useful and logical extension of the recombinant DNA technology, returning the cloned gene to a cell in which its function can be tested.

The present experiment was based on our previous experience with SV40 hybrids carrying both prokaryotic and eukaryotic DNA fragments. Initially, we used an *Escherichia coli* suppressor tRNA gene to develop the SV40 late region deletion vehicle system. We found that such vehicles could be used to propagate the foreign DNA either as virus in productively infected cells[6,7], or as a stable genetic element in transformed or persistently infected cells[8,9]. Subsequently, we inserted a rabbit β-globin coding sequence, derived from a cloned reverse transcript, into two different sites in the SV40 late region[10]. As anticipated, expression of the cDNA gene into a stable, translatable mRNA required the retention of the appropriate signals for mRNA processing (and possibly translation) in the viral vector. A similar conclusion was reached by Mulligan *et al.*[11], who inserted the rabbit sequence at a different site in the viral late region. Most recently, we constructed viruses carrying a portion of the mouse β^{maj}-globin gene, and provided evidence for the use of a globin splice junction and polyadenylation site when the sense strand of the gene was transcribed from the viral late region promoter[12]. However, because of the limitations of our biochemical analyses, we could not be certain that the processing reactions were occurring at exactly the appropriate sites. This encouraged us to insert the complete chromosomal gene into SV40, thereby providing a more stringent test for functional mRNA synthesis—translation into authentic globin.

Construction of an SV40 recombinant carrying the complete chromosomal mouse β^{maj}-globin gene

In the recombinant virus described here, the complete chromosomal mouse β^{maj}-globin gene, including its intervening and adjacent flanking sequences, replaces the viral late gene region. The gene is inserted in the same orientation as the SV40 late protein coding sequences. Therefore, readthrough transcription from the strong viral late region promoter, which is preserved in the vector, generates the globin coding sequence rather than its complement. The construction of this recombinant, depicted in Fig. 1, involved four steps: preparation of the appropriate globin and viral vector DNA segments; ligation *in vitro* of these segments to form linear recombinant molecules with the appropriate orientation; cyclisation of the recombinant genomes *in vivo* following DNA infection of cultured monkey kidney cells; and isolation of a clone of virus having the desired structure.

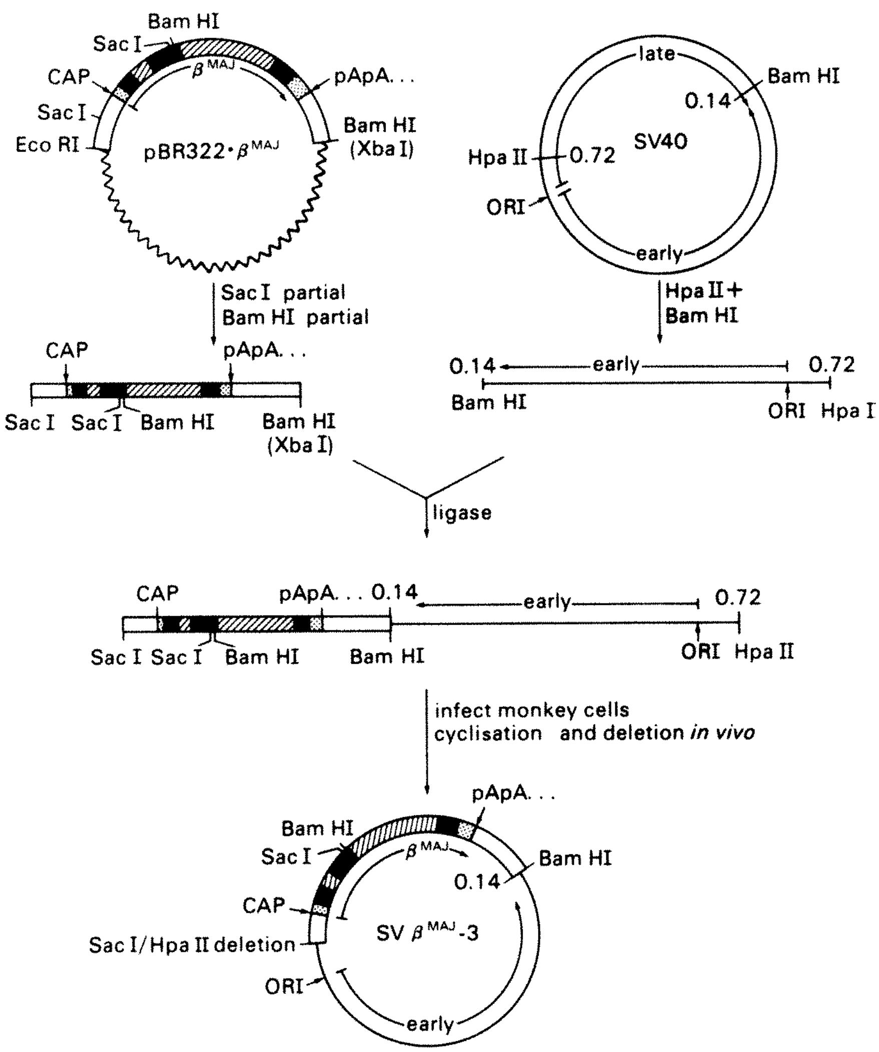

Fig. 1 Construction and propagation of the SV40–mouse β^{maj}-globin gene recombinant. Open bars: mouse β^{maj}-globin gene flanking sequences; stippled bars: globin transcribed, untranslated sequences; solid bars: globin coding sequences; hatched bars: globin intervening sequences; wavy lines: plasmid sequences; solid lines: SV40 sequences. The methods used for DNA preparation, restriction endonuclease cleavage, isolation of fragments on gels, ligation, DNA infection of African green-monkey kidney cells and preparation of virus stocks have been described elsewhere[6,10,12]. **The chromosomal β^{maj}-globin gene fragment:** The upper left diagram shows the structure of pBR322·β^{maj}, which contains the mouse chromosomal β^{maj}-globin DNA sequences from an *Eco*RI site located 1,200 base pairs upstream from the globin cap site to an *Xba*I site located 600 base pairs downstream from the poly(A) addition site. This plasmid was derived from the original recombinant pMB9·β^{maj} (ref. 2) by linking the *Xba*I site to a synthetic dodecamer containing a *Bam*HI site, cloning the resulting *Bam*HI–*Bam*HI fragment in pBR322 (ref. 26), then reconstituting the gene by insertion of the 5′-*Eco*RI–*Bam*HI globin gene fragment. This plasmid was partially digested with *Sac*I and *Bam*HI to generate the 2,400-base pair fragment that was cloned in SV40. **The SV40 vector:** The upper right diagram shows the structure of wild-type SV40 DNA including the direction of transcription of the late and early mRNAs, the origin of viral DNA replication (ORI), and the cleavage sites for *Bam*HI and *Hpa*II relative to the *Eco*RI site at 0.00/1.00 map units[27,28]. The DNA was cleaved with *Bam*HI and *Hpa*II to generate the 3,000-base pair vector fragment. This fragment retains the entire early region, the origin of replication and the sequences corresponding to the 5′- and 3′-termini of the viral late mRNAs; it lacks the coding sequences for the late viral proteins VP1, VP2 and VP3. **The recombinant molecule:** A mixture containing 24 μg of the globin gene fragment and 18 μg of the SV40 vector fragment was treated with DNA ligase, then electrophoresed through a 1% agarose gel to purify the 5,400-base pair linear recombinant genome. This molecule, formed by joining the two *Bam*HI termini, contains the globin coding sequences on the same strand normally occupied by the viral late gene coding sequences. **Propagation:** Approximately 10 ng of the purified recombinant DNA mixed with 70 ng of DNA from the temperature-sensitive early gene mutant helper tsA_{239} and used in a DNA plaque infection of monkey kidney cells at 41 °C. Fifty complementation plaques were picked and used to prepare virus stocks. DNAs from these stocks were immobilised on nitrocellulose filters and tested for hybridisation to a probe containing globin gene 5′-flanking sequences (see Fig. 2 legend). Of three isolates showing unequivocal hybridisation, one was kept for further analysis and designated SVβ^{maj}-3.

We originally cloned the β^{maj}-globin gene in bacteriophage λ as part of a 7,000-base pair fragment of chromosomal DNA[1]. Complete nucleotide sequence analysis of this gene revealed that it is divided into three discontinuous coding segments by two intervening sequences of DNA[5]. The shorter intervening sequence is 116 base pairs long and interrupts the gene between amino acid codons 30 and 31, whereas the longer is 646 base pairs and interrupts between amino acid codons 104 and 105. Because the maximum amount of DNA which can be inserted in the SV40 late region is only 2,500 base pairs, it was not possible to use the whole 7,000-base pair globin fragment. Instead, we prepared a 2,400-base pair subfragment extending from a *Sac*I site, located 430 base pairs upstream from the 5′-end of the globin mRNA sequence, to an *Xba*I site, located 600 base pairs downstream from the 3′-end of the mRNA sequence. This fragment contains, in addition to the entire globin coding sequence, the sequences adjacent to the sites at which the cap is added to the 5′-end and poly(A) to the 3′-end of the globin mRNA. It also contains two RNA splice junctions and their corresponding intervening sequences. To facilitate the preparation of this fragment and its cloning in SV40, we converted the *Xba*I site to a *Bam*HI site by ligation to a synthetic oligonucleotide linker, then subcloned a restriction fragment containing the relevant sequences in a plasmid vector.

The viral vector segment, which was prepared by cleavage of wild-type SV40 DNA with *Bam*HI at map position 0.14 and *Hpa*II at map position 0.72, retains the viral origin of replication, the entire early gene region and the sequences corresponding to the extreme 5′- and 3′-termini of the late 16S and 19S mRNAs. It lacks the coding sequences for the viral late proteins VP1, VP2 and VP3 and the splice junctions for their corresponding mRNAs[13–16]. We have previously demonstrated that various foreign DNA fragments inserted in this vector are efficiently transcribed from the SV40 late promoter, but the resulting transcripts are not appropriately processed and are rapidly degraded in the cell[10]. This might be because the viral late mRNA splice junctions are absent from the hybrid virus. Recombinants formed with this vector lack the viral late genes and are therefore defective. However, because the recombinants retain a functional early gene region, they can be propagated by co-infection with a temperature-sensitive early gene mutant virus as helper[6,7,34]. When such mixed infections are carried out at the non-permissive temperature, progeny virus is produced only by those cells infected with both the recombinant, which provides functional early gene product, and the helper, which provides late gene products.

The globin and viral DNA segments, both of which had one cohesive *Bam*HI terminus, were joined by treatment with DNA ligase to form a linear recombinant molecule that was purified by electrophoresis in an agarose gel. As shown in Fig. 1, the globin gene could join the vector in only one orientation, such that the globin coding sequence is on the strand normally occupied by the viral late protein coding sequences. The resultant recombinant molecule is about 150 base pairs longer than wild-type DNA and has non-complementary termini. However, previous experiments indicated that such molecules could cyclise *in vivo*, and that this is frequently accompanied by the generation of deletions around the junction between the viral and foreign DNAs (D.H.H., unpublished results). Accordingly, the recombinant DNA preparation was mixed with DNA from a temperature-sensitive early gene mutant as helper, and used in a DNA infection of cultured kidney cells. As anticipated, this mixed infection yielded a substantial number of complementation plaques, whereas infection with equivalent amounts of the recombinant or helper DNAs alone did not.

To identify a clone of virus having the desired structure, we prepared virus stocks from these plaques and tested their DNA for hybridisation to a globin probe containing the globin mRNA cap site and the 5′-genomic flanking sequences (see Fig. 2). Of the first 50 plaques tested, three showed unequivocal hybridisation. Presumably, the others had either suffered deletions extending past the 5′-end of the globin gene, or contained only low levels of recombinant virus. One of the positive clones, designated SVβ^{maj}-3, was kept for further analysis. This hybrid multiplies quite efficiently in monkey kidney cells. In our standard conditions of infection, the ratio of intracellular recombinant to helper genome is about 1 : 1.

An analysis of SVβ^{maj}-3 by restriction endonuclease cleavage and gel blot hybridisation (Fig. 2) confirmed that it had the structure expected from its manner of construction and selection. Although the recombinant has the same length as the helper, we were able to purify its DNA because it contains no *Eco*RI sites and one *Sac*I site, whereas the helper contains one *Eco*RI site and no *Sac*I sites. By digestion with the appropriate endonucleases, we showed that the recombinant has deleted 150 base pairs from the 5′-end of the original globin fragment, leaving 280 base pairs of genomic flanking sequence 5′ to the mRNA cap site. Less than 14 base pairs are deleted from the SV40 vector, leaving the predominant viral late mRNA cap sites intact. Thus, SVβ^{maj}-3 contains the complete mouse β^{maj}-globin gene in the sense orientation relative to the viral late region promoter.

Transcription and processing of globin mRNA

Monkey cells infected with SVβ^{maj}-3 efficiently synthesise a stable globin mRNA that is spliced and polyadenylated at the appropriate globin gene sites. However, most of this RNA is initiated at the SV40 late promoter region rather than the globin promoter. Evidence for this is derived from gel transfer hybridisation experiments and the translation studies described below.

To characterise the globin RNA made in infected monkey cells, poly(A)-containing RNA was purified[13,14], denatured[15], electrophoresed through an agarose gel, transferred and covalently attached to diazobenzyloxymethyl paper[16], then hybridised with various ^{32}P-labelled DNA probes to reveal the positions of the separated RNAs (Fig. 3). Hybridisation with total SV40 DNA as probe yielded two bands corresponding to

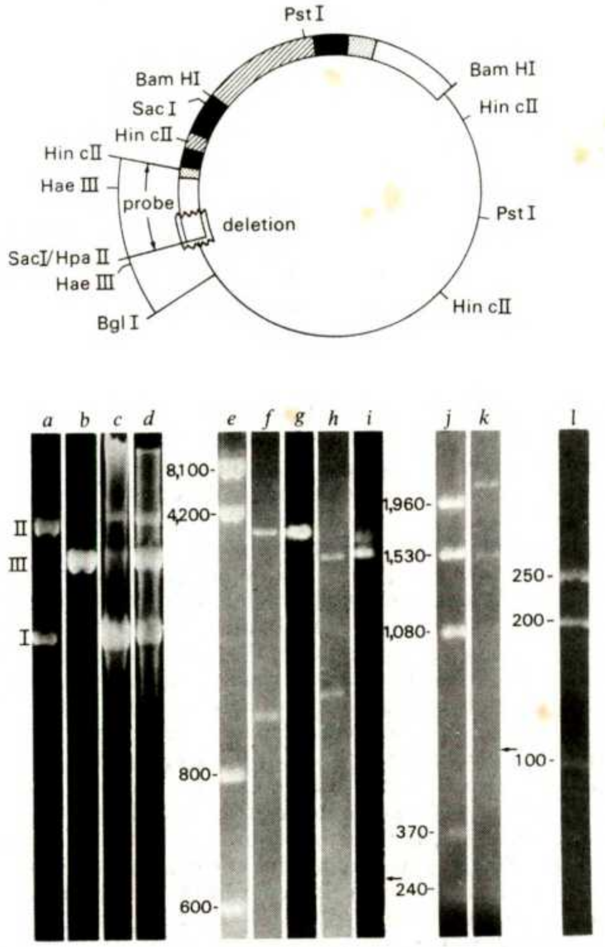

Fig. 2 Structural analysis of the SV40-mouse β^{maj}-globin gene recombinant. The upper diagram is a restriction endonuclease cleavage map of the recombinant virus SVβ^{maj}-3. Solid lines: SV40 sequences; open bars: globin gene flanking sequences; stippled bars: globin gene transcribed, untranslated sequences; solid bars: globin gene coding sequences; hatched bars: globin gene intervening sequences. The bottom panels show an analysis of the recombinant virus by restriction endonuclease cleavage and gel transfer hybridisation. A mixture of SVβ^{maj}-3 recombinant plus *tsA*$_{239}$ helper DNA was prepared by the method of Hirt[29] from monkey kidney cells that had been infected with the recombinant plus helper stock for 48 h at 40 °C. To prepare pure SVβ^{maj}-3 DNA, this mixture was digested with *Eco*RI, which has one site in the helper but none in the recombinant, and the resistant recombinant genomes were recovered as covalently closed circles as shown in track *d*. This preparation was digested with *Sac*I, which has one site in the recombinant but none in the helper, and the linear recombinant molecules were further purified by electrophoresis through a 1% agarose gel. A fragment containing the deleted junction between the globin and SV40 fragments was prepared by digestion with *Hin*cII plus *Bgl*I as shown in the diagram and electrophoresis of the resulting fragments through a 2% agarose gel. Tracks *a–d* were electrophoresed through 1% agarose gels, *e–i* through 1.4% agarose gels, *j*, *k* through 2% agarose gels, and *l* through an 8% acrylamide gel. Tracks *a–f*, *h* and *j–l* are photographs of ethidium bromide-stained gels. Tracks *g* and *i* are autoradiographs of nitrocellulose transfer strips that were hybridised to a pMB9 · βG2/*Sac*I–*Hin*cII probe containing flanking and transcribed, untranslated sequences from the 5′-portion of the globin gene (see upper diagram). Each track represents the following: *a*, undigested wild-type SV40 DNA. The positions of covalently closed circular (form I), nicked circular (form II) and linear (form III) SV40 are shown to the left; *b*, *Eco*RI digest of wild-type SV40 DNA gives linear molecules; *c*, undigested SVβ^{maj}-3 plus *tsA*$_{239}$ DNA; *d*, *Eco*RI digest of SVβ^{maj}-3 plus *tsA*$_{239}$ DNA gives about 50% resistant form I recombinant molecules and 50% sensitive form III helper molecules; *e*, *Sac*I plus *Pst*I digest of pMB9 · βG2 DNA gives marker fragments with lengths (in base pairs) shown to the left; *f*, *Bam*HI digest of SVβ^{maj}-3/*Sac*I DNA gives 3,700- and 1,500-base pair fragments; *g*, transfer of track *f* showing hybridisation to 3,700-base pair fragment; *h*, *Pst*I digest of SVβ^{maj}-3/*Sac*I DNA gives 3,000-, 1,570- and 630-base pair fragments. The position of the 630-base pair fragment, which can be seen only faintly in this reproduction, is indicated by the arrow to the right; *i*, transfer of track *h* showing hybridisation to 3,000-base pair fragment; *j*, *Hin*cII digest of wild-type SV40 DNA gives marker fragments with lengths (in base pairs) shown to the left; *k*, *Hin*cII digest of SVβ^{maj}-3/*Sac*I DNA gives fragments of 2,100, 1,530, 1,080 and 580 base pairs. The position of the 580-base pair fragment, which can be seen only faintly in this reproduction, is indicated by the arrow to the right; *l*, *Hae*III digest of the SVβ^{maj}-3/*Hin*cII–*Bgl*I junction fragment gives three fragments with the lengths (in base pairs) shown to the left. The lengths of these fragments were estimated by their mobility relative to several sequenced SV40 and globin gene fragments (not shown). There are single *Hae*III sites in the globin gene sequence between the *Hin*cII site and the deleted *Sac*I site and in the SV40 sequence between the *Bgl*I site and the deleted *Hpa*II site. Because *Hae*III digestion of the junction fragment yields three fragments, both *Hae*III sites must be preserved. The *Hae*III site in SV40 is 15 base pairs from the *Hpa*II site. Therefore, the deletion must cover less than 14 base pairs of SV40 sequence and 150–164 base pairs of globin gene flanking sequence.

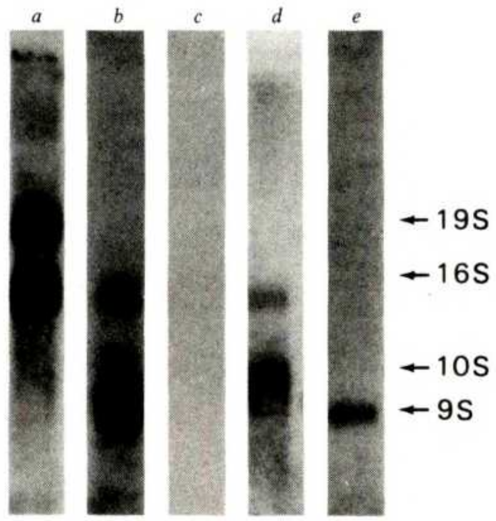

Fig. 3 Gel transfer hybridisation analysis of the globin RNA made in monkey cells infected with the SV40–mouse β^{maj}-globin gene recombinant. **RNAs and hybridisation:** A confluent monolayer of about 2×10^7 monkey kidney cells was infected with 5 ml of the SVβ^{maj}-3 plus $\underline{tsA_{239}}$ stock and incubated for 48 h at 40 °C. Total cell poly(A)-containing RNA was prepared by extraction with hot phenol[13] and oligo(dT)–cellulose chromatography[14], denatured with glyoxal[15], electrophoresed through a 1.5% agarose gel and transferred to diazobenzyloxymethyl paper[16]. Each lane contains 10% of the total sample. The resulting imprints were preincubated, hybridised with approximately 0.1 ng of ^{32}P-labelled, nick-translated DNA probe (25–50 c.p.m. per pg), washed and autoradiographed[10,16]. Globin mRNA was prepared from reticulocytes obtained from anaemic mice. **Probes** (see maps, Figs 1, 2): The globin probes were prepared by cleavage of the plasmid pMB9 · MβG2, which contains the entire 7,000-base pair chromosomal mouse β^{maj}-globin fragment[2]. The globin coding sequence probe contained 900 base pairs extending from the *Pst*I site 75 base pairs upstream from the 3′-coding segment to the *Xba*I site 600 base pairs downstream from the poly(A) addition site. The globin intervening sequence probe contained 600 base pairs extending from the *Bam*HI site 18 base pairs upstream from the large intervening sequence to the *Pst*I site 75 base pairs upstream from the 3′-coding segment. Control experiments with globin mRNA show that hybridisation of the 18 base pairs of coding sequence included in this intervening sequence fragment would not be detected in our conditions of hybridisation, washing and autoradiography. Wild-type SV40 DNA was prepared as described previously[6]. The SV40 late mRNA leader probe contained 250 base pairs extending clockwise from the *Bgl*I site at 0.67 map units in the *Hpa*II site at 0.72 map units. Each track represents the following: *a*, SVβ^{maj}-3 RNA hybridised with total SV40 DNA probe; *b*, SVβ^{maj}-3 RNA hybridised with globin coding sequence probe; *c*, SVβ^{maj}-3 RNA hybridised with globin intervening sequence probe; *d*, SVβ^{maj}-3 RNA hybridised with SV40 late mRNA leader sequence probe; *e*, globin mRNA (10 ng) hybridised with globin coding sequence probe.

the viral late 16S and 19S mRNAs, presumably due to transcription of the helper virus. When a fragment containing globin coding sequences was used as the probe, we detected a strong globin RNA band at approximately 10S, indicating molecules up to 300 bases longer than authentic globin mRNA (800 bases). This RNA did not hybridise with a probe derived from the large globin intervening sequence, indicating that it has been eliminated and that the second globin splice junction functioned properly in the infected cells. Because the globin RNA is efficiently translated into mouse β^{maj}-globin (see below), it is clear that the first splice junction is also functional and that both splicing reactions are accurate. To determine whether this RNA was initiated at the globin gene promoter or at the upstream viral promoter, we used as probe an SV40 fragment containing the viral late mRNA leader sequences. This probe hybridised strongly to the ~10S globin RNA band, indicating that most of these molecules arise from transcription initiation at the viral promoter. This may also explain the length heterogeneity of the globin RNA as the 5′-viral leader segments are known to be quite variable in length[17,18]. The SV40 leader probe also detected a 16S band and a very faintly hybridising 19S band (not visible in the reproduction of Fig. 3, lane *d*) which probably represents helper virus transcription.

In addition to the ~10S globin RNA, using the coding sequence probe we routinely detected a 16S (1,600 base) band comprising about 15% of the total globin-specific RNA. This RNA is at least partially spliced as shown by its failure to hybridise to the globin large intervening sequence probe. We suspect, but have not proved, that this species results from readthrough past the globin polyadenylation site to the downstream viral polyadenylation site. A similar situation was encountered in our more detailed studies of a recombinant carrying a segment from the 3′-portion of the mouse β^{maj} gene[12].

Judging from the intensities of the bands shown in Fig. 3, we estimate that the steady-state level of the mouse globin RNA produced in monkey cells compares favourably with that of viral late mRNA. Assuming that the globin and late viral mRNAs are initiated at the same rates from the SV40 late promoter, this indicates that the globin RNA is as stable as late viral mRNA. We estimate that approximately 5% of the total cell poly(A)-containing RNA is globin RNA, representing roughly 200,000 molecules per cell.

Mouse β^{maj}-globin is synthesised by infected monkey cells

Monkey kidney cells infected with SVβ^{maj}-3 synthesise a protein which we have identified as mouse β^{maj}-globin by immunoprecipitation, two-dimensional electrophoresis and tryptic fingerprint analysis. These tests indicate that the mouse globin mRNA is accurately spliced in monkey cells, and that the mouse gene translational signals are functional.

Figure 4 (lanes *a–h*) shows an experiment in which monkey cells were infected with either the SVβ^{maj}-3 plus helper stock or with no virus or wild-type SV40 as controls, incubated until late in the lytic cycle, then labelled for 1 h with ^{35}S-methionine. Sonic extracts were prepared, and a portion of each was electrophoresed through a polyacrylamide-SDS gel, which separates proteins according to their molecular weight[19]. As might have been anticipated from the fact that SV40 does not shut down host cell protein synthesis, the resulting autoradiograms showed a complex variety of proteins in which the globin region of the gel was obscured by host bands. However, when the cell proteins were first immunoprecipitated with purified goat anti-mouse β-globin antibody, we detected a band with the same mobility as authentic mouse globin in the SVβ^{maj}-3-infected cell extract but not in the mock or wild-type SV40-infected cell extracts. (The additional bands seen in these lanes were also seen using anti-SV40 T-antigen serum, and apparently result from artefactual trapping during the immunoprecipitation procedure.) The exact co-migration of this band with authentic mouse β^{maj}-globin was confirmed by a mixing experiment (Fig. 4, lane *h*).

The identity of this protein as mouse β^{maj}-globin is supported by a two-dimensional electrophoresis experiment (Fig. 4, lanes *i–m*). Extracts were prepared and immunoprecipitated as described above, unlabelled mouse globin was added to the eluted proteins and the mixtures were submitted to non-equilibrium *p*H gradient gel electrophoresis, which separates proteins according to their isoelectric points[20]. When the proteins co-migrating with mouse β^{maj}-globin, identified by staining, were eluted and electrophoresed through a polyacrylamide-SDS gel, we detected a strong globin band in the SVβ^{maj}-3-infected preparation but not in the mock or wild-type SV40-infected preparations. A mixing experiment (Fig. 4, lane *m*) confirmed the co-migration of this band with authentic globin. Note that the non-equilibrium *p*H gradient gels used in this experiment are capable of separating proteins that differ by only a single charge unit[20].

Tryptic fingerprint analysis provided further evidence that the protein encoded by SVβ^{maj}-3 is mouse β^{maj}-globin. The globin band from SVβ^{maj}-3-infected cells was purified by immunoprecipitation and polyacrylamide-SDS gel electrophoresis, digested with trypsin and submitted to two-dimensional chromatography on a cellulose thin layer plate[21,22]. The resulting autoradiogram (Fig. 5) showed two spots with the same relative positions as those found in a digest of authentic mouse

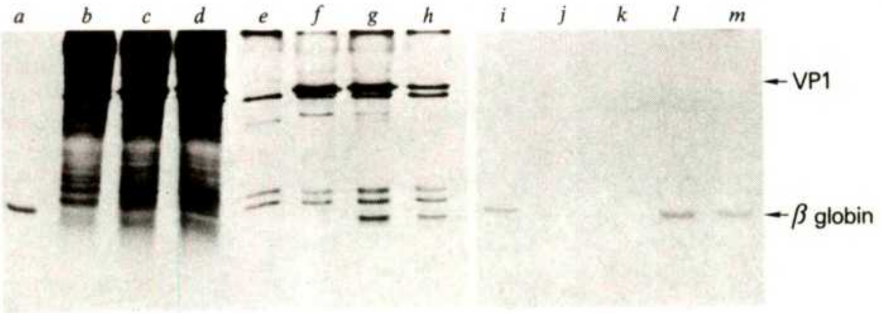

Fig. 4 Production of mouse β^{maj}-globin in monkey cells infected with the SV40-mouse β^{maj}-globin gene recombinant. **Proteins:** Confluent monolayers of about 2×10^7 monkey cells were treated with 1 ml of medium, wild-type SV40 or the SVβ^{maj}-3 plus tsA_{239} stock and incubated for 48 h at 40 °C. The cells were washed three times with methionine-free medium, then labelled for 1 h with 200 μCi of ^{35}S-methionine (516 Ci mmol^{-1}, NEN) in 1 ml of methionine-free media. After washing twice in ice-cold phosphate-buffered saline (PBS), the cells were scraped off the plates in PBS, collected by centrifugation, resuspended in 0.8 ml of PBS containing 1% Triton X-100 and 0.5% sodium deoxycholate, sonicated for 1 min at full power in a Heat Systems sonicator, and centrifuged at 12,000*g* for 30 min. The supernatants were recovered and analysed as described below. To prepare authentic mouse β^{maj}-globin, Friend erythroleukaemia cells were induced with 1% dimethyl sulphoxide for 3 d, labelled with 25 μCI ml^{-1} ^{35}S-methionine for 12 h, then lysed by freeze-thawing. Haemoglobin was purified on a carboxymethyl cellulose column and converted to globin by precipitation with 2% HCl in acetone[30]. Pure β^{maj}-globin chains were prepared by carboxymethyl cellulose column chromatography in the presence of 8 M urea[31]. **Immunoprecipitation:** 10 μl of heparin (10 mg ml^{-1}), 10 μl of normal goat serum and 50 μl of a 10% suspension of heat-killed formalin-fixed *Staphylococcus aureus* Cowen I bacteria were added to 500 μl of cell extract[32]. Following 30 min on ice, the bacteria were removed by centrifugation, the supernatant was added to a predetermined excess of purified goat anti-mouse β-globin antibody and incubated 12 h at 4 °C. Then, 50 μl of *S. aureus* suspension was added, the mixtures were kept at 4 °C for 1 h and the bacteria collected by centrifugation. The pellet was washed three times in PBS containing 1% Triton X-100 and 0.5% deoxycholate. A 20% aliquot of each sample was resuspended in 25 μl of SDS sample buffer[19], boiled for 5 min, centrifuged to remove the bacteria, then analysed by polyacrylamide-SDS gel electrophoresis. The remainder of each sample was resuspended in 100 μl of non-equilibrium *p*H gradient electrophoresis gel sample buffer[20], mixed with 5 μg of unlabelled mouse globins, incubated for 1 h at room temperature, centrifuged to remove the bacteria, then analysed by two-dimensional electrophoresis. **Gel electrophoresis:** 20% polyacrylamide-SDS gels were prepared, run and fluorographed as described elsewhere[19,33]. Non-equilibrium *p*H gradient gels[20] were prepared in 0.6 × 10 cm tubes and electrophoresed at 400 V (constant voltage) for 3 h. The gels were soaked in 50% methanol for 24 h, stained in 0.01% Coomassie brilliant blue, 50% methanol and 12.5% acetic acid for 15 min, then destained in 7% methanol and 1.25% acetic acid for 18 h. The region of the gel containing mouse β^{maj}-globin (well separated from β^{min}- and α-globin) was excised and the proteins eluted by electrophoresis, precipitated with 2% HCl in acetone, resuspended in 50 μl of SDS sample buffer, boiled for 5 min and electrophoresed through a polyacrylamide-SDS gel. **Quantification of globin synthesis:** 0.67% of the trichloroacetic acid-precipitable radioactivity in the extract from SVβ^{maj}-3-infected cells was recovered in the immunoprecipitate. Of this, 15% was in the globin band as determined by densitometry of the autoradiogram (track *g*). Thus, 0.67% × 5% = 0.1% of the ^{35}S-methionine incorporated during the 1-h pulse was found in globin. Tracks *a*–*h* are one-dimensional, polyacrylamide-SDS gel analyses of total cell extracts and immunoprecipitates. Tracks *i*–*m* are the second dimension from the two-dimensional analyses (non-equilibrium *p*H gradient gel electrophoresis followed by polyacrylamide-SDS gel electrophoresis) and were run on a separate polyacrylamide-SDS gel. Each track represents the following *a*, mouse β^{maj}-globin; *b*, total mock-infected cell extract; *c*, total wild-type SV40-infected cell extract; *d*, total SVβ^{maj}-3-infected cell extract; *e*, immunoprecipitate of mock-infected cell extract; *f*, immunoprecipitate of wild-type SV40-infected cell extract; *g*, immunoprecipitate of SVβ^{maj}-3-infected cell extract; *h*, mixture of mouse β^{maj}-globin and immunoprecipitate of SVβ^{maj}-3-infected cell extract (one-half the amounts applied to tracks *a* and *g* were mixed after immunoprecipitation and elution); *i*, mouse β^{maj}-globin; *j*, mock-infected; *k*, wild-type SV40-infected; *l*, SVβ^{maj}-3-infected; *m*, mixture of mouse β^{maj}-globin and SVβ^{maj}-3-infected samples (one-half the amounts applied to tracks *i* and *l* were mixed after immunoprecipitation elution).

β^{maj}-globin prepared from induced Friend erythroleukaemia cells. The identity of the peptides from the SVβ^{maj}-3 protein with authentic β^{maj}-globin was confirmed by fingerprinting a mixture containing equal amounts of radioactivity from both samples. Presumably, the two spots detected represent the two internal methionine-containing tryptic peptides of β^{maj}-globin[23]. The globin gene also encodes an N-terminal methionine which is removed in globin-producing cells. Because only the expected two spots were observed in the SVβ^{maj}-3 protein digest, we conclude that the monkey cells have appropriately removed the N-terminal methionine. Note also that one of the methionine-containing peptides (Leu 105–Lys 121) lies within the third coding sequence immediately after the second splice junction (Arg 104–Leu 105). This provides further evidence for accurate splicing of the mouse globin mRNA in monkey cells.

We estimate that the mouse β^{maj}-globin accounts for approximately 0.1% of the ^{35}S-methionine incorporated during a 1-h pulse (see Fig. 4 legend). This is close to the synthesis rates for the viral proteins VP2 and VP3. Thus, the globin mRNA seems to be translated about as efficiently as viral mRNA in infected monkey kidney cells.

Distribution of RNA processing enzymes

Although the ubiquity of the enzymes and factors involved in translation has long been recognised, relatively little is known about the species and cell-type distribution of RNA processing enzymes. Our previous data[12] and experiments presented here establish that monkey kidney cells can both splice and polyadenylate mouse globin RNA. Although we have not characterised the polyadenylation reaction in detail, we believe that the splicing reaction is both specific and efficient. This is indicated by the translation of the resulting mRNA into β^{maj}-globin, and by the failure to observe accumulation of unspliced precursor molecules.

Because mice and monkeys diverged over 70 Myr ago, and because kidney fibroblast cells are not in the erythroid cell lineage, we suspect that the RNA splicing and polyadenylation signals are widely recognised among mammalian species and cell types. Our findings are inconsistent with the notion that the differentiation of a globin-producing cell requires the production of enzymes that specifically process globin and other erythroid mRNAs. On the other hand, the results support the validity of comparing the sequences of globin genes from divergent organisms so as to define the nucleotide sequence specificity of RNA processing enzymes.

SV40 transducing viruses as genetic tools

We have established that a mammalian chromosomal gene can be expressed in a foreign cell after cloning in SV40. The chief difference between this and earlier experiments by Mulligan *et al.*[11] and ourselves[10] using cDNA recombinants is that here expression of the globin gene depends on the mouse genomic signals for RNA splicing and polyadenylation rather than on the corresponding viral signals. Hence, it seems to be a useful substrate for determining the effects of nucleotide sequence alterations on RNA processing. Further, although we have used the SV40 late promoter in this recombinant, it should be possible to bring the globin gene under the control of its own promoter by inverting the mouse fragment relative to the viral vector.

Although cell transformation techniques may also be useful for globin gene transfer experiments (refs 24, 25 and R. Axel, personal communication), there are several advantages of SV40 as a vector for this purpose. First, its small size simplifies localised biochemical mutagenesis. Second, it replicates efficiently, reaching levels up to 100,000 copies per cell late in infection, thus simplifying the detection of gene products encoded by the transduced DNA. Finally, by placing the genomic fragment under the optional control of the strong viral late region promoter, transcription of the inserted DNA can be assured even if its own promoter is not functional. The system's major limitation is that at most 2,500 base pairs of foreign DNA

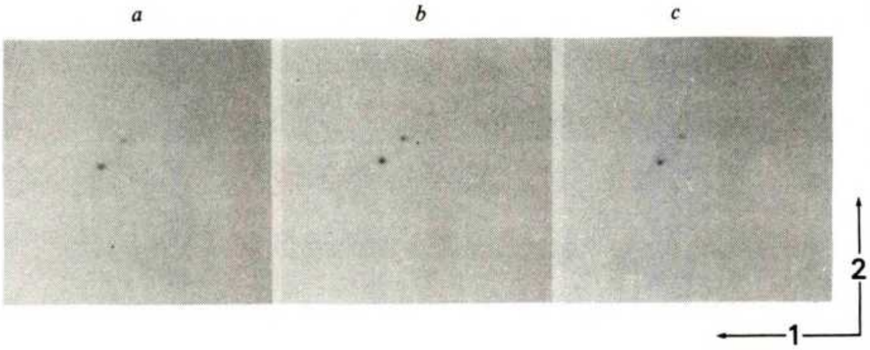

Fig. 5 Fingerprints of tryptic peptides. A 1-ml aliquot of SVβ^{maj}-3-infected cell extract was immunoprecipitated as described in Fig. 4 legend, eluted in SDS sample buffer, mixed with 5 μg of unlabelled mouse globin and electrophoresed through a 20% polyacrylamide-SDS gel. A sample of authentic ^{35}S-methionine-labelled mouse β^{maj}-globin from induced Friend cells was electrophoresed in a separate track. The globin bands were identified by staining, excised, washed with 10% methanol, then digested *in situ* with trypsin-TPCK (Worthington)[21]. The supernatants were lyophilised, resuspended in 25 μl of H_2O and analysed by two-dimensional ascending chromatography on 20 × 20 cm cellulose thin-layer plates (Brinkman). The first solvent was isoamyl alcohol/pyridine/H_2O (90:120:105)[22]. The second solvent was butanol/pyridine/acetic acid/H_2O (32.5:25:5:20) containing 7% (w/v) 2,5-diphenyloxazole[21]. The autoradiographs were exposed for 8 d at −70 °C. *a*, Globin from SVβ^{maj}-3-infected cells; *b*, authentic mouse β^{maj}-globin; *c*, mixture of globin from SVβ^{maj}-infected cells and authentic mouse β^{maj} globin (one-half the amounts used in *a* and *b*).

can be inserted into the late region of the recombinant if it is to be encapsidated into virions. This obstacle might be overcome by propagating the hybrid genome as an episome in persistently infected monkey cells[9], thus overcoming the requirement for encapsidation. Furthermore, it is clear that, although we have concentrated on the globin system, the approach should also be applicable to a variety of other genes.

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Rabbit β-globin mRNA production in mouse L cells transformed with cloned rabbit β-globin chromosomal DNA

Ned Mantei, Werner Boll & Charles Weissmann

Institut für Molekularbiologie I, Universität Zürich, 8093 Zürich, Switzerland

Mouse thymidine kinase-negative L cells were transformed with a cloned rabbit chromosomal β-globin gene linked to the cloned thymidine kinase gene of herpes simplex virus type 1. Most thymidine kinase-positive cell lines contained one or more copies of rabbit β-globin DNA and produced up to 2,000 copies of rabbit β-globin RNA per cell indistinguishable from its authentic counterpart. No mouse β-globin mRNA was detected.

RECOMBINANT DNA technology has made it possible to isolate, amplify and determine the primary structure of any eukaryotic DNA segment of interest. To explore the functional significance of particular regions of a genome, it is desirable to generate localised modifications in the nucleic acid and then evaluate the resulting changes in the biological properties of the genome, *in vitro* or *in vivo* (see ref. 1). The reintroduction of defined nucleic acid segments, in particular of cloned DNA, into eukaryotic cells has been accomplished by various methods. Mechanical microinjection of RNA or DNA into oocytes[2,3] or into somatic cells[4] delivers a large dose of nucleic acid to a relatively small number of identifiable cells. Biophysical and biochemical approaches use liposomes[5,6] or erythrocytes[7] loaded with RNA which are fused with the target cells, or direct treatment of target cells with nucleic acid in the presence of facilitating agents[8–11]. Efficient delivery of DNA to a large population of cells is attained by the use of DNA incorporated into viral particles[12].

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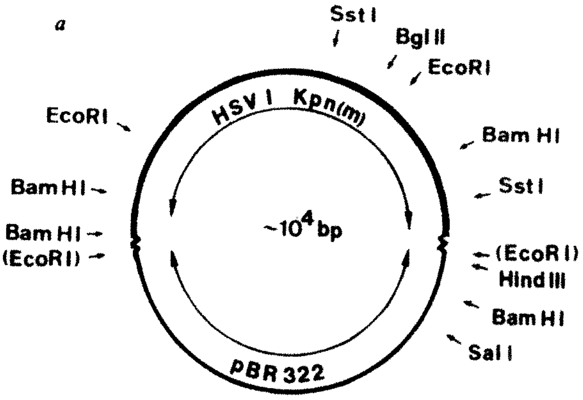
When a high proportion of the treated cells take up and retain the administered DNA the experiment can be evaluated by assaying the entire cell sample; this is the case when oocytes are individually injected, or when cells are infected with viral particles containing a hybrid SV40 genome. Where the foreign DNA transforms only a minute proportion of the cells, as is the case in most procedures involving administration of exogenous naked DNA, a selection procedure must be used to enrich for transformed cells.

Thymidine kinase-negative cells acquire thymidine kinase activity after transformation with herpes virus DNA, or even better, with an appropriate, purified restriction fragment[9]. As



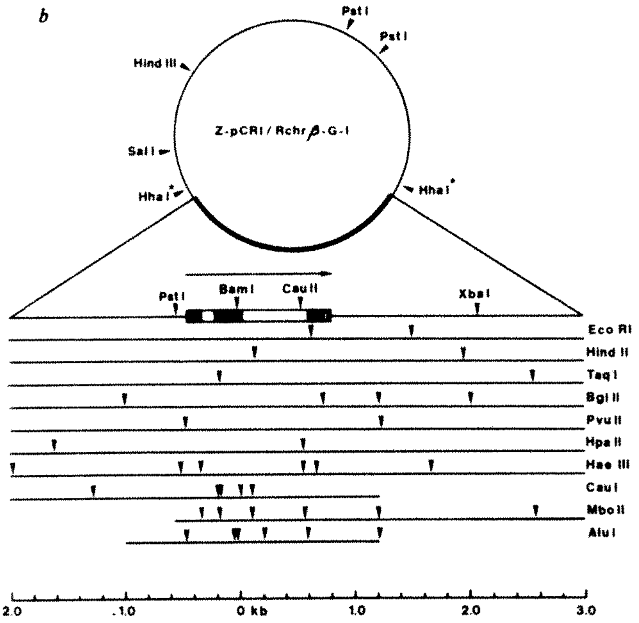


Fig. 1 The restriction map of a hybrid plasmid containing a herpes simplex virus type I DNA fragment with the thymidine kinase gene (*a*) and a rabbit β-globin chromosomal DNA fragment (*b*). The restriction sites of the rabbit β-globin chromosomal DNA hybrid Z-pCRI/RchrβG-1 (ref. 16) were mapped from the single *Bam*HI site by the procedure of Smith and Birnstiel (ref. 42 and A. van Ooyen, J. van den Berg, N.M. and C.W., unpublished results). The thymidine kinase DNA-containing hybrid plasmid Z-pBR322/HSV · TK-M4 was prepared by cleaving herpes simplex virus type 1 DNA with *Kpn*I, elongating the fragments with dAMP and joining them to *Eco*RI-cleaved, TMP-elongated pBR322 (refs 14, 15). Restriction sites were mapped from the single *Bgl*II site by the procedure of Smith and Birnstiel[41] and by total cleavage. The *Eco*RI sites in parentheses are obliterated.

Table 1 Rabbit β-globin DNA and β-globin mRNA content of transformed mouse L cell lines

DNA used for transfection β-globin	DNA used for transfection Thymidine kinase	No. transformants / No. dishes	Cell line	Approximate no. rabbit β-globin DNA copies per cell*	Approximate no. rabbit β-globin RNA transcripts per cell† Expt 1	Expt 2
—	TK-P1	83/2	P1/S-1	0	<20	
—			P1/S-2	0	<20	
—	TK-M2	82/2	M2/S-1	NT	<20	
			M2/S-2	0	<20	
—	TK-M4	78/2	M4/S-1	NT	<20	
			M4/S-2	0	<20	
RβG	TK-P1	71/4	P1/R-1	0	NT	
			P1/R-2	1	NT	
			P1/R-3	3	300	600
			P1/R-4	8	300	50
			P1/R-5	1	50	400
			P1/R-6	2	<20	
			P1/R-7	6	<20	
			P1/R-8	1	100	
RβG	TK-M2	59/5	M2/R-1	20	2,000	2,000
			M2/R-2	0.25	NT	
			M2/R-3	1	300	
			M2/R-4	8	<20	
			M2/R-5	0.25	200	
			M2/R-6	0.25	200	
			M2/R-7	3	200	
			M2/R-8	3	<20	
RβG	TK-M4	14/4	M4/R-1	1	150	
			M4/R-3	8	200	
			M4/R-5	0	<20	
			M4/R-6	0.25	NT	
			M4/R-7	1	400	

Thymidine kinase-negative mouse L cells (LMTK$^-$) were cultured in Dulbecco's modified Eagle's medium (Flow), 10% calf serum (Eurobio) and 30 μg ml^{-1} bromodeoxyuridine (BUdR). Before transfection the cells were cultured for three doublings in the absence of BUdR, then plated at 4×10^5 cells per 9-cm dish 24 h before DNA addition. TK-gene-containing plasmid DNA was prepared in P-2 containment conditions. To prepare concatenated DNA for transfection, 1.25 μg TK-gene plasmid (Z-pBR322/HSV · TK-P1, -M2 or -M4 (refs 14, 15) and 6.25 μg Z-pCR1/RchrβG-1 (ref. 16) were digested with *Sal*I (New England Biolabs), heated for 10 min at 65°C to inactivate the enzyme, and mixed into a final volume of 0.1 ml with 70 mM Tris-HCl (*p*H 7.2), 10 mM $MgCl_2$, 10 mM dithiothreitol and 1 mM ATP. Ligation was with T_4 DNA ligase for 3 h at 16 °C; no monomeric DNA was detectable by agarose gel electrophoresis. After phenol extraction, ether extraction and ethanol precipitation, the DNA was mixed with 80 μg salmon sperm DNA and used for transfection of five dishes of LMTK$^-$ cells according to the protocol of Wigler *et al.*[17]. Control cells were transfected with *Sal*I-cleaved TK-gene plasmid, without ligation. After 19 days in modified HAT[13] medium (15 μg ml^{-1} hypoxanthine, 1 μg ml^{-1} aminopterin, 5 μg ml^{-1} thymidine in Dulbecco's modified Eagle's medium), colonies were picked with cloning rings and grown up for analysis (see Figs 2, 4 and 5).

* The β-globin DNA content of 10–30 μg of total cell DNA was estimated visually from the autoradiogram of Fig. 2 and additional similar experiments.

† The β-globin RNA content was estimated visually from the autoradiograms of Weaver–Berk–Sharp analyses. Expt 1: the probe used was [5'-^{32}P]-labelled *Bgl*II-cleaved PβG (data not shown). Expt 2: from the experiment of Fig. 4*A*. Calculations are explained in the text. NT, not tested.

only cells possessing thymidine kinase activity can survive and grow in a medium containing hypoxanthine, aminopterin and thymidine (HAT medium)[13], it is possible to select from among 10^6–10^7 cells the few tens or hundreds of cells which were transformed by DNA containing the thymidine kinase gene[9]. We have used this system to select mouse cells transformed with cloned rabbit chromosomal β-globin DNA linked to a cloned herpes simplex virus type 1 (HSVI) DNA fragment containing the thymidine kinase (TK) gene. We find that 19 of 21 cell lines selected in HAT medium contain rabbit β-globin DNA, and 12 of 16 such lines synthesise rabbit β-globin-specific RNA which is indistinguishable from natural β-globin mRNA in all aspects tested.

Transformation of TK$^-$ mouse cells with rabbit β-globin DNA linked to HSVI–TK DNA

We have prepared hybrid plasmids consisting of pBR322 linked to a fragment of HSVI DNA containing the TK gene, either a *Bam*HI (Z-pBR322/HSV · TK-P1) or a *Kpn*I fragment (Z-pBR322/HSV · TK-M2 and Z-pBR322/HSV · TK-M4)[14,15]. Figure 1 shows the restriction maps of the hybrid plasmid Z-pBR322/HSV · TK-M4 (*a*) and of the hybrid Z-pCRI/RchrβG-1 (*b*, abbreviated to RβG), which consists of pCRI linked to a β-globin chromosomal rabbit DNA fragment[16]. The plasmids containing β-globin and thymidine kinase DNA were cleaved at their single *Sal*I sites and joined at a molar ratio of 2.5:1 by T$_4$ DNA ligase to form concatenates. TK$^-$ L cells were transformed with the concatenates (1.5 μg total DNA per 8×10^5 cells) using the calcium phosphate method[8] as described by Wigler *et al.*[17]. Cells transformed in parallel with a *Sal*I-cleaved thymidine kinase plasmid alone served as a control. After 19 days in HAT-containing medium 8–47 colonies per dish were recovered (Table 1). Each of 27 colonies was expanded to about 6×10^7 cells, and DNA and RNA were isolated.

Identification of rabbit β-globin DNA in thymidine kinase-transformed mouse L cells

The DNA from the thymidine kinase-transformed cell lines was cleaved with *Eco*RI and *Pst*I, denatured, subjected to agarose gel electrophoresis and transferred to nitrocellulose filters by the procedure of Southern[18]. As reference, the chromosomal β-globin DNA hybrid RβG, cleaved with the same enzymes, was run in parallel. The filter was hybridised with a ^{32}P-labelled, cloned rabbit β-globin cDNA probe devoid of plasmid sequences[19]. As shown in the restriction map of Fig. 1, cleavage of rabbit chromosomal DNA by *Pst*I and *Eco*RI is expected to yield two β-globin-specific fragments of 1,200 and 900 base pairs, respectively. The autoradiogram (Fig. 2) shows that the four DNA preparations from cells transformed with the rabbit β-globin–TK DNA concatenates had two bands with the same mobilities and the same relative intensities as the 900- and 1,200-base pair bands derived from the β-globin chromosomal plasmid. Altogether, 19 of 21 TK-positive cell lines contained β-globin DNA by this analysis. The control, DNA from cells transformed with a TK plasmid only, did not show these characteristic bands. From the intensities of the bands of *Eco*RI- and *Pst*I-cleaved cell DNA relative to those of known amounts of similarly treated cloned chromosomal DNA run in parallel, we estimate visually that the cell lines P1/R-3, P1/R-4, P1/R-5 and M2/R-1 contain about 3, 8, 1 and 20 copies of rabbit β-globin DNA per cell (Table 1).

Identification and characterisation of rabbit β-globin-specific transcripts from transformed mouse L cells

In vivo transcription of the extraneous rabbit β-globin gene was monitored by a modification of the Berk–Sharp procedure[20] developed by Weaver *et al.*[21], as exemplified in Fig. 3. The hybrid plasmid RβG contains a *Bgl*II site immediately following the 3′-end of the β-globin coding sequence. The hybrid was cleaved with *Bgl*II and labelled with ^{32}P at the 5′-termini. After digestion with *Pst*I, the *Pst*I–*Bgl*II (1,291/1,299) fragment, labelled at the *Bgl*II site (on the (−)strand), was isolated. The labelled DNA was denatured, hybridised with the RNA sample in 80% formamide, treated with nuclease S$_1$ and analysed by polyacrylamide gel electrophoresis in 7 M urea. Only if the labelled 5′-terminus of the DNA is protected by RNA complementary to it is a radioactive fragment recovered. As the probe is labelled on the (−)strand, only (+)strand RNA is detected. Mature rabbit β-globin mRNA protects a fragment about 134 nucleotides long (from the *Bgl*II site to the 3′-end of the large intervening sequence (intron)), whereas a precursor containing both introns would protect about 1,200 nucleotides and a precursor containing only the large intron would protect 929 nucleotides.

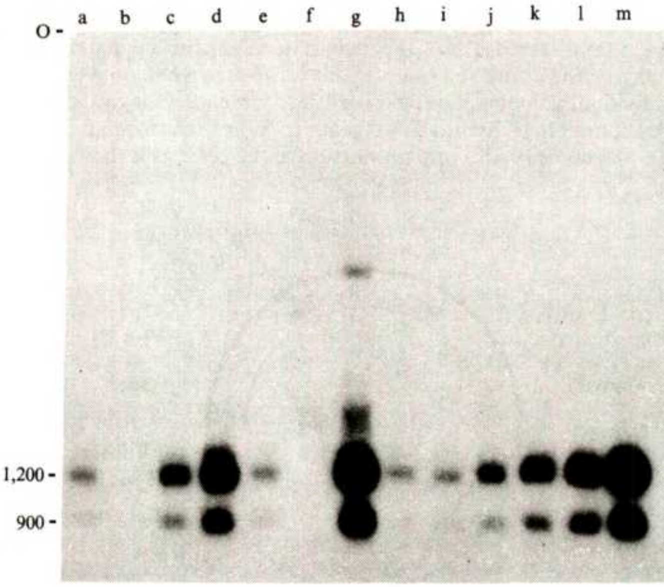


Fig. 2 Detection of chromosomal rabbit β-globin sequences in DNA of transformed mouse L cell lines. Mouse L cell lines transformed with the rabbit β-globin hybrid DNA linked to a thymidine kinase plasmid, or with a thymidine kinase plasmid only, were obtained as described in Table 1 legend. 1.5×10^7 cells were lysed with 1.1% SDS in 0.15 M NaCl and 0.1 M EDTA (*p*H 8), extracted with CHCl$_3$ and isoamyl alcohol (24:1), and the DNA spooled during addition of ethanol to 66%. After treatment with pancreatic RNase, 20 μg ml^{-1} in 10 mM NaCl, 0.5 mM EDTA and 5 mM Tris-HCl (*p*H 7.5) for 8 h at 37 °C, overnight incubation at 37 °C with 100 μg ml^{-1} pronase in 100 mM NaCl, 5 mM EDTA and 50 mM Tris-HCl (*p*H 7.5) and phenol extraction, the DNA was collected by spooling as above. About 300–600 μg of DNA were recovered. Cell DNA (10 μg) was digested with *Pst*I (MRC, Porton) and *Eco*RI (MRC, Porton), denatured and subjected to electrophoresis on a 1.4% agarose gel as described by Jeffreys and Flavell[43]. The DNA was transferred to a 15 × 17 cm Millipore membrane[18] and hybridised to a ^{32}P-labelled rabbit β-globin probe (specific activity, 8×10^7 c.p.m. per μg; about 325 ng in 25 ml) as described[43]. The probe was obtained as follows. The rabbit β-globin cDNA-containing hybrid plasmid PβG[44] was cleaved with nuclease S$_1$ in formamide[45], joined to *Hin*dIII linkers[46] and recloned in the *Hin*dIII site of pBR322 (ref. 19). A hybrid plasmid containing the β-globin sequence present in PβG was identified by nucleotide sequence analysis (Z-pBR322 · H3/RcβG-4.13) and prepared in quantity. After cleaving with *Hin*dIII, the β-globin-specific fragment was purified by agarose gel electrophoresis and nick-translated[47] using [α-^{32}P]-labelled dATP and dCTP (400 Ci mmol^{-1} each; NEN, Dreieich). Autoradiography of the filter was for 30 days. Lanes b and f, DNA from cell lines P1/S-2 and M2/S-2, transformed only with a *Sal*I-cleaved TK-gene plasmid. Lanes c, d, e and g, DNA from cell lines P1/R-3, P1/R-4, P1/R-5 and M2/R-1, transformed with the rabbit β-globin containing hybrid RβG, linked to a TK-gene plasmid. Lanes a and h–m contain as reference RβG digested with *Pst*I and *Eco*RI: 1.6, 1.6, 1.6, 3.2, 6.4, 12.8 and 32 pg of DNA hybridisable to the probe (1.8 pg is the equivalent of one β-globin gene). Lanes a, h and j–m also contain 10 μg of DNA from cell line P1/S-2 digested with *Pst*I and *Eco*RI as carrier. The mobility of the 900- and 1,200-base pair bands is about 1% less in the presence of 10 μg total DNA per slot (compare lanes h and i) than in its absence. O, origin. Ordinate in base pairs.

The probe discriminates between mouse and rabbit globin mRNA because the rabbit mRNA has a *Bgl*II recognition

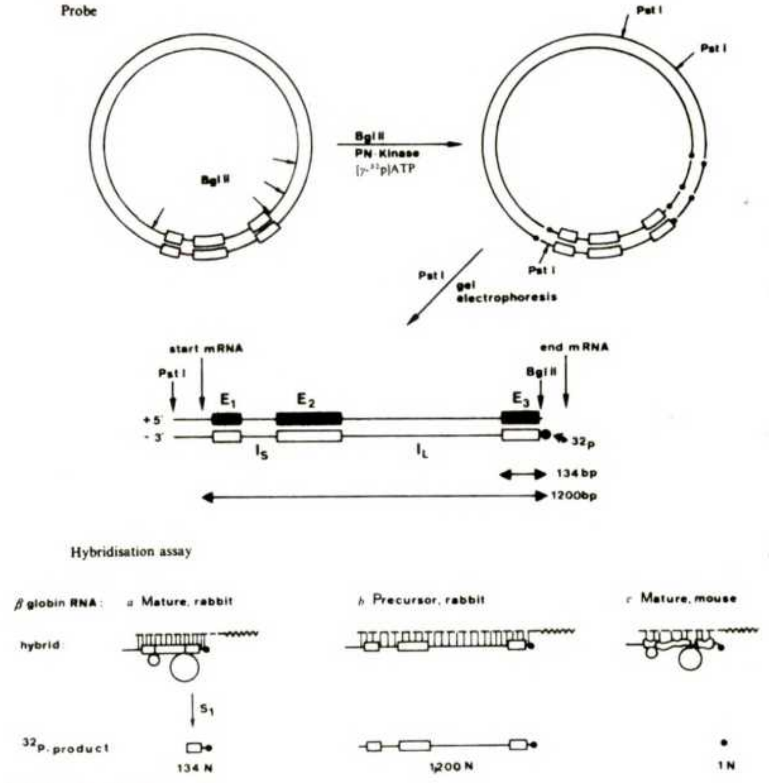


Fig. 3 Application of the Weaver–Berk–Sharp method to distinguish mature and precursor rabbit β-globin mRNA in the possible presence of mouse β-globin mRNA. The procedure is a modification of the method of Berk and Sharp[20]. The probe is a DNA restriction fragment ^{32}P-labelled at one 5'-terminus (rather than being uniformly labelled). The denatured DNA is hybridised to the RNA sample in 80% formamide, digested with S_1 nuclease, denatured and analysed by polyacrylamide gel electrophoresis in 7 M urea. Only if the 5'-terminus of the probe was hybridised to RNA is a labelled DNA fragment recovered. The *Pst–Bgl*II ((+)strand 1,291/(−)strand 1,299 bases) fragment derived from the rabbit chromosomal β-globin plasmid (see Fig. 1), labelled at the *Bgl*II site, is diagnostic for (+)strand β-globin sequences and allows the distinction between rabbit precursor RNA, rabbit mature RNA and mouse RNA. E, exon; I_s and I_L, small and large intron, respectively. PN·Kinase, polynucleotide kinase.

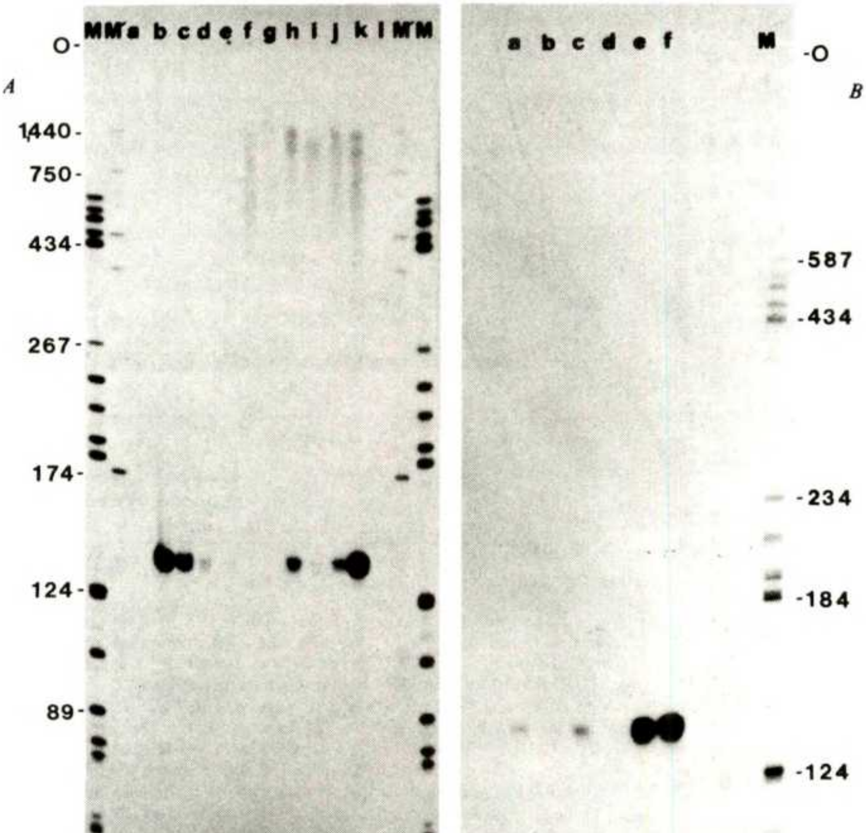


Fig. 4 Detection and characterisation of rabbit β-globin RNA in transformed mouse L cells by the Weaver–Berk–Sharp method. Total cell RNA was prepared from about 10^8 cells as described elsewhere[29]. The yield was about 1.6 mg per 10^8 cells. The ^{32}P-labelled probe (described in Fig. 3) was prepared by cleaving 20 µg Z-pCRI/RchrβG-1 with *Bgl*II (New England Biolabs), treating with bacterial alkaline phosphatase (BAPF, Worthington) and labelling with polynucleotide kinase (P-L Biochemicals) and [γ-^{32}P]ATP (specific activity 3,300 Ci mmol^{-1}, NEN) as described elsewhere[48]. After purification on a Sephadex G-50 column, the DNA was cleaved with *Pst*I (MRC, Porton), and the labelled 1,291–1,299-base pair fragment isolated after electrophoresis through a 1.5% agarose gel and passed through a Chelex column. About 25 ng of probe (13,000 c.p.m.) were denatured by heating in 80% formamide for 15 min at 100 °C and mixed with 50 µg RNA. Hybridisation was carried out in 10 µl of 0.4 M NaCl, 0.04 M PIPES (*p*H 6.4), 0.001 M EDTA and 80% formamide, at 57 °C for 16 h under paraffin oil. The sample was diluted with 100 µl 0.25 M NaCl, 0.03 M sodium acetate (*p*H 4.5) and 0.002 M $ZnSO_4$ and digested with 50 units of S_1 nuclease (purified by the procedure of Wiegand *et al.*[49]). After $CHCl_3$ extraction to remove the paraffin oil, and precipitation with ethanol, the samples were dissolved in 5 µl 90% formamide, 0.05% bromphenol blue and 0.05% xylene cyanol FF, heated for 10 min at 100°C, and analysed by electrophoresis through a 0.1 × 28 × 40 cm 5% polyacrylamide gel (acrylamide : bis-acrylamide, 30:1) in 0.089 M Tris base, 0.089 M boric acid, 2.5 mM EDTA and 7 M urea. Autoradiography was carried out with use of an Ilford Fast Tungstate intensifier screen at −70 °C for 2–5 days. *A*, Unfractionated RNA samples. Lanes a–e, 0, 3, 1, 0.3 and 0.1 ng rabbit globin mRNA ($\alpha + \beta$). Lanes f, g, RNA from cell lines P1/S-1 and P1/S-2, which had been transformed only with a *Sal*I-cleaved TK-gene plasmid. Lanes h–k, RNA from cell lines P1/R-3, P1/R-4, P1/R-5 and M2/R-1, transformed with the rabbit β-globin DNA-containing hybrid linked to a TK-gene plasmid. Lane l, 50 ng mouse globin mRNA. Lanes M, M', [5'-^{32}P]-labelled fragments of plasmid pBR322 as size markers[50]. *B*, Poly(A)$^+$ and poly(A)$^-$ fractions of RNA samples. RNA (50 µg) from cell line M2/R-1 or rabbit globin mRNA (3 ng) was passed through a 0.1 ml oligo(dT) cellulose (type 7, P-L Biochemicals) column in 0.2 M NaCl, 0.01 M EDTA, 0.1% SDS and 0.1 M Tris-HCl (*p*H 7.5)[51]. The column was washed with the same buffer and eluted with H_2O. The RNA in the flow-through and wash (poly(A)$^-$) and in the H_2O eluate (poly(A)$^+$) was precipitated with ethanol. Lanes a, b, poly(A)$^+$ and poly(A)$^-$ fractions of M2/R-1 RNA; lanes c, d, poly(A)$^+$ and poly(A)$^-$ fractions of rabbit globin mRNA. Lanes e, f, 20 and 40 µg unfractionated rabbit bone marrow RNA. Lane M, as above; O, origin. Ordinate in base pairs.

sequence[22] whereas mouse mRNA does not (ref. 23 and N.M., unpublished results), and therefore does not protect the labelled 5′-terminus of the probe. Figure 4*A* shows that as little as 0.1 ng rabbit β-globin mRNA (lane e) gave the expected 134-nucleotide fragment whereas 50 ng mouse globin mRNA (lane l) gave no such band. The RNA from transformed cell lines P1/R-3, P1/R-4, P1/R-5 and M2/R-1 (lanes h–k) yielded as major product the 134-nucleotide fragment. Thus, the majority of globin-specific transcripts are partly or entirely devoid of the large intron. Some weak bands corresponding to fragments of 750–1,200 nucleotides may be due to unprocessed or incompletely processed precursor molecules. RNA from cells transformed with only a thymidine kinase plasmid (lines P1/S-1 and P1/S-2, lanes f and g) gave no detectable bands. From the amount of cell RNA added to the assay (50 μg), the amount of RNA per cell (about 25 pg)[24] and the fact that the intensity of the 134-nucleotide band is similar to that given by 3 ng of total rabbit globin mRNA (about half of which is β-specific), we estimate that cell line M2/R-1 contains about 2,000 rabbit β-globin RNA molecules per cell. By the same criteria, P1/R-3, P1/R-4 and P1/R-5 contain about 600, 50 and 400 β-globin transcripts per cell. As relative band intensities were estimated visually, these values as well as those in Table 1 are only approximate. Altogether, 12 of 16 globin DNA-containing clones had β-globin-specific transcripts (see Table 1).

To determine whether the small intron was present, RNA was hybridised to RβG DNA which had been digested with *Bam*HI, labelled at the 5′-termini and cleaved again with *Bgl*II. After S_1 cleavage and denaturation, polyacrylamide gel electrophoresis revealed a 212-nucleotide fragment, in the case of both authentic rabbit β-globin mRNA and RNA from samples P1/R-3, P1/R-4, P1/R-5 and M2/R-1 (Fig. 5). This shows that the small intron was not present in the transcripts, because otherwise a 480-nucleotide fragment would have appeared.

The 5′-terminus of the β-globin-specific transcripts was mapped by the same procedure as before, using as probe a restriction fragment extending from the *Mbo*II cleavage site at position 129/128 to the *Pst*I site at position −95/−99, [5′-^{32}P]-labelled at the *Mbo*II-cleaved end. As shown in Fig. 6, natural rabbit β-globin mRNA gave rise to a labelled fragment 128 nucleotides long; RNA from the cell line M2/R-1 gave rise to a strong and P1/R-3 to a weaker band in the same position as the β-globin mRNA; in the case of RNA from cell lines P1/R-4 and P1/R-5, only very weak bands were discernible on the original films (but not on the photograph), perhaps because of the relatively low content of β-globin-specific RNA in these cell lines (Table 1). This experiment showed that the only detectable 5′-terminus of the β-globin transcripts mapped in the same position as that of authentic β-globin mRNA; however, we cannot exclude the possibility that a certain proportion of molecules may have a variety of 5′-termini which escaped detection because of a broad distribution of the resulting bands.

The presence of poly(A) on the β-globin-specific transcripts from transformed L cells was demonstrated by fractionating RNA from M2/R-1 on an oligo(dT) column and assaying separately the flow-through fraction [poly(A)$^-$] and the fraction retained at high salt [poly(A)$^+$], using as probe the *Pst*I–*Bgl*II (1,291/1,299) fragment 5′-labelled at the *Bgl*II terminus. About 80% of the β-globin transcript was detected in the poly(A)$^+$ fraction (Fig. 4B).

The mobility of the rabbit β-globin-specific RNA present in the cell line M2/R-1 was compared with that of natural rabbit globin mRNA using the 'Northern transfer' procedure of Alwine *et al.*[25]. The RNAs were subjected to electrophoresis on an agarose gel in denaturing conditions, transferred to diazobenzyloxymethyl paper and hybridised to ^{32}P-labelled rabbit β-globin cDNA[19]. As shown in Fig. 7, poly(A)$^+$ RNA derived from 150 μg of M2/R-1 RNA gave an autoradiographic band with the same mobility and the same intensity as about 8 ng of authentic rabbit globin mRNA; no band was found with the poly(A)$^+$ RNA from 150 μg of RNA from cells transformed with TK DNA only.

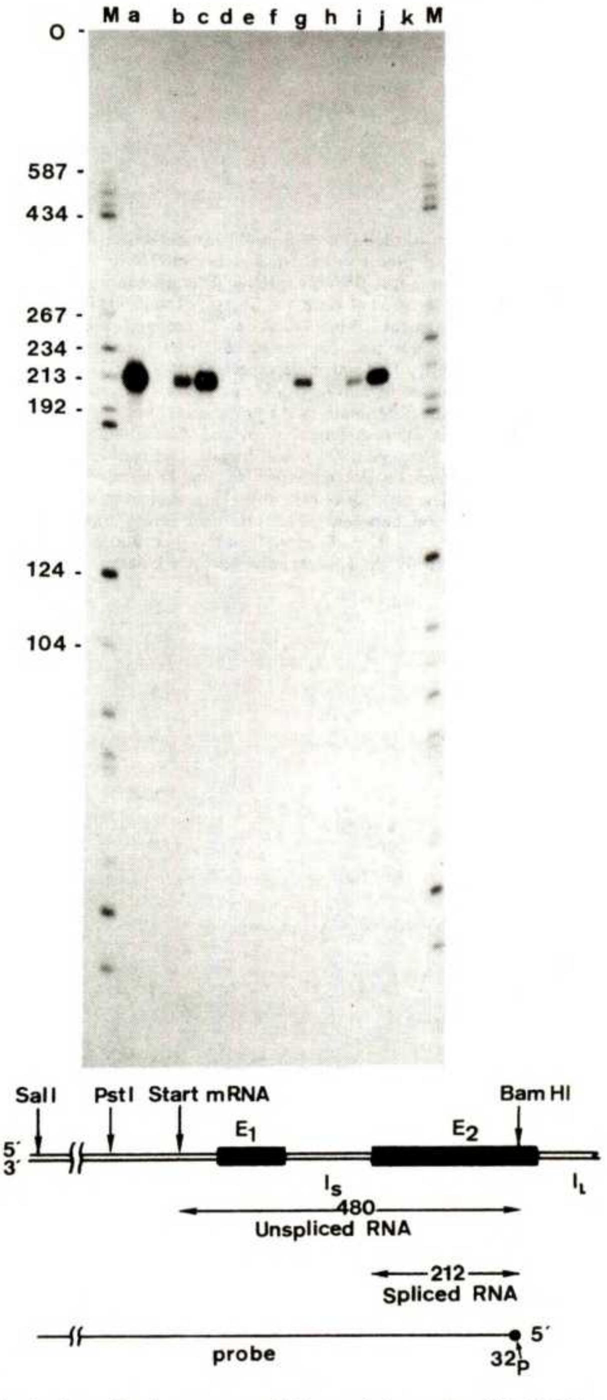


Fig. 5 Assay for the presence of the small intron in rabbit β-globin transcripts from transformed mouse cells. The RNA preparations are those described in Fig. 4 legend. The probe was prepared by digesting Z-pCRI/RchrβG-1 with *Bam*HI, labelling the 5′-terminus with ^{32}P-phosphate (specific activity 2,100 Ci mmole^{-1}) and cleaving with *Bgl*II. Lanes a–c, 3, 0.3 and 1.1 ng rabbit globin RNA ($\alpha+\beta$). Lane d, 50 ng mouse globin RNA. Lanes e, f, RNA from cell lines P1/S-1 and P1/S-2, transformed only with a *Sal*I-cleaved TK-gene plasmid. Lanes g–j, RNA from cell lines P1/R-3, P1/R-4, P1/R-5 and M2/R-1, transformed with the rabbit β-globin DNA-containing hybrid linked to a TK-gene plasmid. Lane k, no RNA. M, Size marker: pBR322 digested with *Bsp*I and [5′-^{32}P]-labelled.

Finally, we determined whether transformed L cells produce mouse β-globin mRNA, in particular when they are synthesising rabbit β-globin mRNA. We again used the Weaver–Berk–Sharp assay, with the [5′-^{32}P]-labelled 123-nucleotide *Bsp*–*Bsp* (−) strand fragment of cloned mouse β-globin cDNA[26] as probe. This (−)strand fragment, which corresponds to the mouse β-globin mRNA segment extending from nucleotide 222 to 344, was purified by hybridising it to mouse β-globin mRNA, isolating the hybrid on an oligo(dT) column and recovering the DNA after alkaline hydrolysis. The assay was carried out as described above; 0.1 ng of mouse β-globin mRNA gave a strong positive signal whereas 10 ng rabbit globin mRNA gave no band, as a sequence complementary to the ^{32}P-labelled terminus of the probe is not present on the rabbit β-globin mRNA. RNA (50 μg total) from L cell lines transformed with TK DNA only (P1/S-1, P1/S-2) or with rabbit β-globin–TK DNA concatenates (P1/R-3, P1/R-4, P1/R-5, M2/R-1) gave no detectable signal (data not shown). From this experiment we estimate that the transformed L cell lines tested, in particular that with 2,000 molecules rabbit β-globin mRNA per cell (M2/R-1), contained less than 50 strands of mouse β-globin mRNA per cell.

Discussion

Concatenates containing a high ratio of β-globin DNA to TK DNA proved to be a convenient device for introducing into mouse L cells multiple β-globin gene copies together with a selective marker which permitted the isolation of transformed cells. Previous experience[27] suggests that the exogenous DNA is largely integrated into host cell sequences, but this point requires further investigation.

Most of the cell lines transformed with rabbit β-globin DNA produced rabbit β-globin-specific RNA. It is striking that the cell line M2/R-1, which contained the highest number of gene copies per cell (about 20), also contained the highest number of RNA molecules (2,000 per cell); however, this correlation did not hold throughout.

The rabbit β-globin-specific RNA of line M2/R-1 was examined in closest detail and was found to be indistinguishable from natural mRNA with regard to its electrophoretic mobility, its 5′-terminus, the absence of the large and small introns and the presence of a 3′-terminal poly(A) tail. The β-globin RNAs of the other cell lines, when tested for one or the other of the criteria, had similar properties.

Synthesis of β-globin mRNA in the mouse occurs through a short-lived 15S precursor[28–32] which represents a complete transcript of the gene, including the introns[33–35]. The same is apparently true for the rabbit β-globin mRNA[36]. It is clear from our results that the splicing enzyme(s) of the mouse cell is capable of acting effectively on the rabbit β-globin transcript. A comparison of the nucleotide sequences of the rabbit and mouse β-globin genes had shown that although the internal sequences of the homologous introns were very different[16,53], the homologous intron–exon junction regions were very similar. The fact that the DNA of SV40, a monkey virus, when injected into *Xenopus* oocytes, leads to the formation of apparently normal SV40 proteins shows that the splicing systems of even widely divergent species are compatible[37]. However, yeast seems to be incapable of splicing the rabbit β-globin transcript[38].

Ziff and Evans[39] have shown that in the case of the adenovirus late mRNA, the cap-carrying 5′-terminus represents the initiation site of transcription. In the case of mouse β-globin RNA the 15S precursor and the mature mRNA have the same 5′-terminal sequence[21] and the same cap structures[26]. If, as we believe, the 15S β-globin precursor is the primary transcript (but for a contrary view, see ref. 31), we may conclude that the rabbit β-globin RNA produced in transformed mouse cells (at least in cell lines P1/R-3 and M2/R-1) originates at the true promoter. We are not certain whether other 5′-termini occur at lower levels, and we cannot yet exclude the possibility that the transcript is initiated upstream at a plasmid or a host promoter, and that the correct, capped 5′-terminus arises by processing. In this connection it is interesting that β-globin transcripts generated in yeast transformed with chromosomal β-globin DNA have 5′-termini which map downstream from the correct 5′-terminus, and 3′-termini mapping within the large intron[38].

No mouse β-globin mRNA was found in TK- or β-globin DNA-transformed mouse L cells used in our experiments (<50 molecules per cell), even when they contained 2,000 rabbit

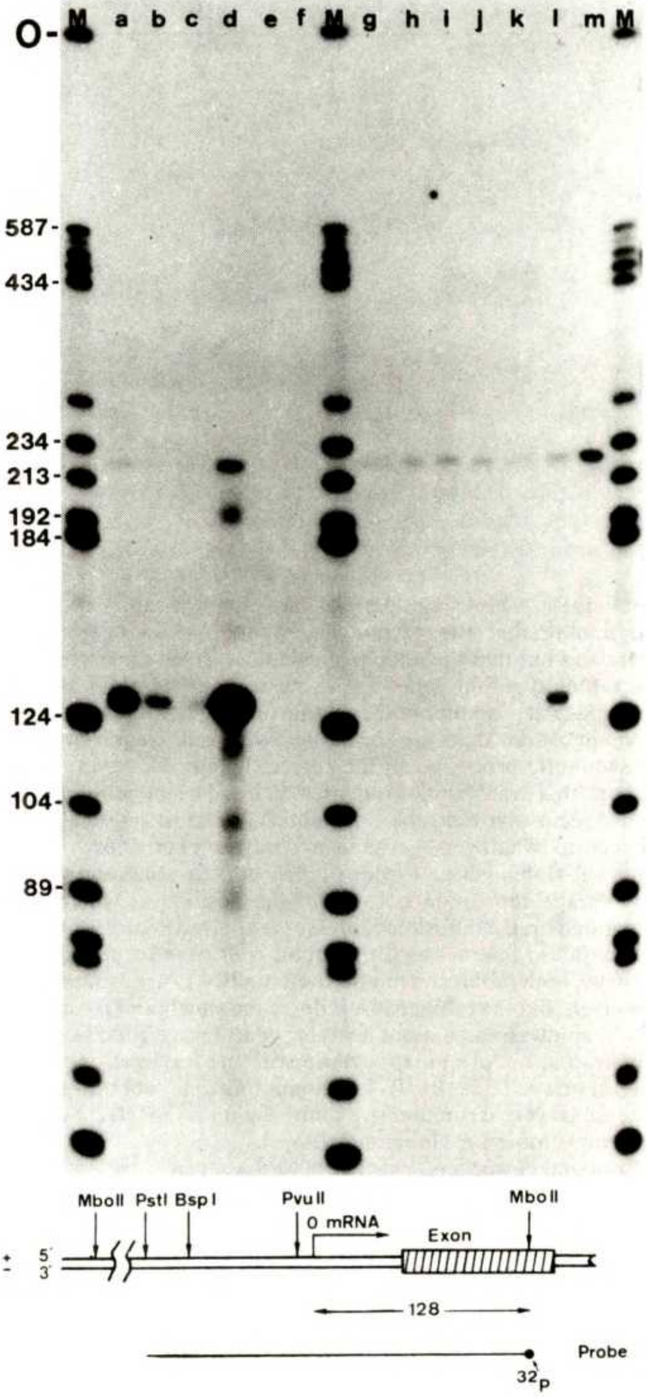


Fig. 6 Mapping of the 5′-terminus of rabbit β-globin-specific transcripts from transformed mouse L cells. The RNA preparations are those described in Fig. 4 legend. The probe for mapping the 5′-end was prepared as follows. Plasmid Z-pCRI/RchrβG-1 was digested with *Hha*I and the largest fragment isolated by centrifugation through a 5–23% sucrose density gradient in a Spinco SW41 rotor, at 30,000 r.p.m. for 12 h at 15 °C. The 6.4-kilobase *Hha*I fragment was cleaved with *Mbo*II (New England Biolabs), and labelled with polynucleotide kinase and [γ-^{32}P]ATP (specific activity, 3,200 Ci mmol^{-1}). After cleavage with *Pst*I, the 223-base pair fragment was isolated by electrophoresis through a 1.6% agarose gel. Lanes a–c, 3, 1 and 0.3 ng rabbit globin mRNA. Lane d, 20 μg rabbit bone marrow RNA. Lane e, no RNA. Lane f, 50 ng mouse globin mRNA. Lanes g, h, RNA from cell lines P1/S-1 and P1/S-2, transformed only with a *Sal*I-cleaved TK-gene plasmid. Lanes i–l, RNA from cell lines P1/R-3, P1/R-4, P1/R-5 and M2/R-1, transformed with the rabbit β-globin DNA-containing hybrid linked to a TK-gene plasmid. Lane m, DNA probe not digested with S_1 nuclease. M, Size marker: pBR322 digested with *Bsp*I and [5′-^{32}P]-labelled.

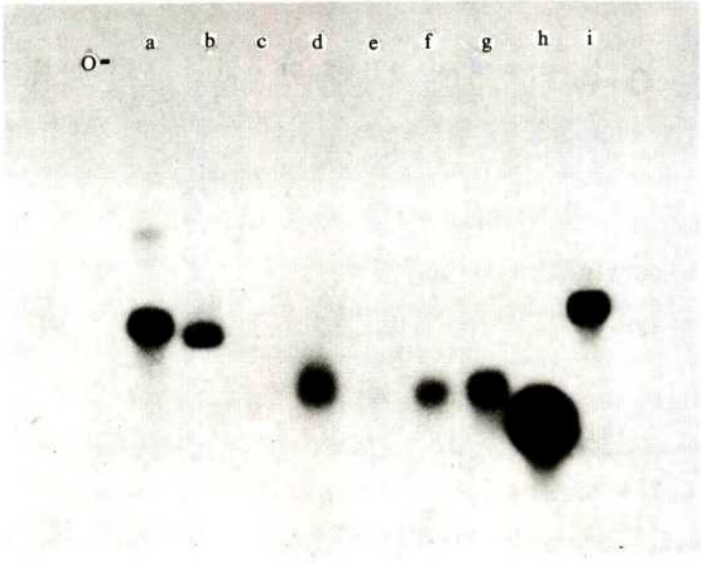


Fig. 7 The electrophoretic mobility of rabbit β-globin transcripts from transformed mouse L cells. Poly(A)$^+$ RNA was prepared as described in Fig. 4 legend. β-Globin mRNA and denatured restriction fragments of rabbit β-globin-specific DNA were used as size markers. All samples (10 μl) were mixed with 40 μl of a mixture consisting of 100 μl 0.1 M sodium phosphate buffer (*p*H 7), 200 μl glyoxal (Merck, Darmstadt; passed three times through BioRad AG-501-X8 CD) and 500 μl dimethyl sulphoxide (Fluka), and heated at 50°C for 60 min[52]. Electrophoresis was through a 14 × 10 × 0.5 cm 1.5% agarose gel in 10 mM sodium phosphate (*p*H 7). The nucleic acid was transferred to diazobenzyloxymethyl paper as described by Alwine *et al.*[25] and hybridised with the ^{32}P-labelled β-globin DNA probe (1.6×10^8 c.p.m. per μg) described in Fig. 2 legend. (a) RβG DNA digested with *Pst*I and *Bgl*II (1,299 base pairs). (b) RβG DNA digested with *Pvu*II and *Bgl*II (1,209 base pairs). (c) Poly(A)$^+$ RNA from 150 μg of P1/S-1 cells (transformed with TK DNA only). (d) Poly(A)$^+$ RNA from 150 μg of M2/R-1 cells (transformed with rabbit β-globin DNA and TK DNA). (e–g) Rabbit β-globin mRNA 1, 4 and 8 ng, respectively. (h) Cloned rabbit β-globin cDNA (*Hin*dIII-cleaved Z-pBR322 · H3/RcβG-4.13, see Fig. 2 legend; ~540 base pairs). (i) RβG cleaved with *Pst*I and *Bgl*II (1,299 base pairs). All samples contained 20 μg yeast RNA as carrier. O, Origin.

globin mRNA molecules per cell. The simplest explanation for the finding that the extraneous rabbit β-globin genes are expressed but that the endogenous mouse genes are not is that the rabbit β-globin genes are being transcribed from a non-physiological promoter (either within or outside the rabbit DNA segment), and that the resulting oversized transcripts are subsequently processed to the correct length. However, if we assume that transcription initiates at the correct promoter, it would seem that the rabbit promoter is at most only partially subject to whatever mechanism controls expression of the mouse β-globin genes: if control is *cis* effective the rabbit gene may escape repression because of the position at which it is integrated or, if a diffusible repressor is operative (*trans* control) it may fail to interact with the rabbit control sequence. Alternatively, both rabbit and mouse β-globin RNAs are produced at low levels, but the rabbit RNA is degraded slowly and the mouse RNA rapidly. Explanations invoking gene dosage effects such as the titrating-out of a putative repressor[40] are less likely, because at least one cell line, P1/R-5, contains only one rabbit β-globin gene and yet accumulates rabbit β-globin mRNA but no detectable mouse β-globin mRNA.

Berg and coworkers[12] have replaced a segment of the SV40 late region by a cloned cDNA copy of rabbit β-globin mRNA. Monkey cells infected by such hybrid DNA underwent a lytic cycle, during which the DNA was greatly amplified. Hybrid mRNA molecules containing the β-globin sequence were transcribed from an SV40 promoter, leading to the accumulation of $>10^4$ hybrid mRNA molecules per cell (P. Berg, personal communication); β-globin was efficiently and accurately translated. We have not yet analysed the proteins produced in our transformed mouse cells, but it seems reasonable to assume that β-globin synthesis, if it occurs, will bear some relationship to the β-globin mRNA content of the cell, and will thus be substantially lower than in the lytic SV40 hybrid system. Thus, thymidine kinase-linked transformation will probably be useful for the study of initiation of transcription and splicing and the SV40 hybrid system for the investigation of translation.

After this manuscript was submitted Wigler *et al.*[41] reported co-transformation of mouse L cells with rabbit β-globin DNA and HSVI–TK DNA.

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letters

Absorption spectrum of the accretion column in VV puppis

N. Visvanathan

Mount Stromlo and Siding Spring Observatories, Research School of Physical Sciences, The Australian National University

D. T. Wickramasinghe

Applied Mathematics Department, The Australian National University, Canberra ACT 2601, Australia

VV Puppis is an AM Herculis type binary system exhibiting variable circular and linear polarisation[1]. In its 'normal' state, the optical light curve shows a slow rise to maximum ($m_{max} \sim 15$–16) and a rapid drop to minimum ($m_{min} \sim 17.5$–18) where it remains for ~60% of its 100-min period[2–4]. However, the system has also been observed in faint inactive states[5] ($m_{min} \sim m_{max} \sim 15$). Only a few spectroscopic observations of VV Pup have been published and these show a continuous spectrum with emission lines of hydrogen and helium superimposed. We present here spectrophotometric observations obtained on 2 February 1979. Broad absorption features are seen for the first time in this object in the optical spectrum during the bright phases with high circular polarisation. We suggest that these features be identified with the 6th, 7th and 8th harmonics of cyclotron absorption in a magnetic field of ~3×10^7 G.

The observations were obtained using the image dissector scanner[8] modified as a polarimeter at the $f/15$ Cassegrain focus of the 4-m Anglo-Australian telescope. Full details of the scanner–polarimeter system which is similar to that described by Nordsieck[9] will be given elsewhere.

We obtained 12 scans of VV Pup from 11.48 UT to 14.00 UT with an effective time resolution of 8 min. The spectra cover the wavelength region λλ3,500–7,000 Å and the spectral resolution is ~10 Å. The visual V magnitude calculated from each reduced scan is plotted in Fig. 1 against the phase of VV Pup computed according to Walker's[2] ephemeris. The absolute energy distributions for the bright and faint phases are displayed in Fig. 2. The computed mean (λλ4,000–6,000 Å) value of circular polarisation during the bright phase at 11.58 UT is ~+4.0%, and during the bright phase at 13.40 UT is ~+6.0%.

On the basis of the shape of the optical light curve, (Fig. 1) our observations apparently correspond to an epoch in which VV Pup was in its 'normal' state. However, two important differences should be noted. First, the phase of eclipse ingress differs by $\Delta\phi \sim 0.2$ relative to Walker's ephemeris. This drift in phase is large compared to the maximum drift recorded previously and it could be the result of the period of enhanced mass transfer in March 1978, or a similar event which has occurred more recently. Second, the spectra obtained during the bright phases show broad absorption features covering the spectral regions λλ4,100–4,600, 4,600–5,400, 5,400–6,200, which have not been previously observed.

Variable circular and linear polarisation observed in VV Pup supports a model in which the primary is a strongly magnetic white dwarf rotating synchronously with the binary period[10,11]. In suitable conditions the material from the late type secondary will be channelled towards the magnetic poles where in general a shock ($T \sim 10^8$ K) will form[12,13]. In this model the variable optical radiation originates from a small hot spot at one of the magnetic poles of the white dwarf. The bright epochs with $m_{min} \sim m_{max} \sim 15$ correspond to a situation when the second pole also is active. The faint epochs with $m_{min} \sim m_{max} \sim 18$ occur when both poles are inactive.

In the framework of the above model our observations with $m_{min} \sim 17.8$ and $m_{max} \sim 15.7$–16.5 (see Fig. 1) correspond to an epoch when only the first pole was active. The observed sign of circular polarisation and lack of circular polarisation during minimum phase are consistent with this hypothesis. The absorption features, which are seen only during the bright phases, are probably associated with the gas at the base of the accretion column near the active magnetic pole. From brightness temperature considerations alone, a Zeeman interpretation due to hydrogen at fields $\geqslant 10^8$ G seems unlikely. An attractive possibility is that these features are cyclotron absorption harmonics arising from the accretion column which is believed to dominate the optical radiation.

The wavelength of the mth harmonic for cyclotron absorption by electrons in a magnetic field B (G) is given by $\lambda_m = 1/m\ (10^8/B)$ 10,710 Å. The observed features can thus be identified with the 6th (5,800 Å), 7th (4,977 Å) and 8th (4,380 Å) harmonics with $B = 3.08\times10^7$ G. There is also marginal evidence for the fifth (6,960 Å) harmonic. As the observed features are broad, and do not have well defined cores, there is an uncertainty of 1 harmonic in the above identifications with a corresponding uncertainty of ~20% in B. In principle the detection of lower harmonics in the IR will enable a precise determination of B. However, the comparison star is likely to dominate the spectrum of long wavelengths and the detection of low harmonics may not be possible.

An upper limit to the temperature of the radiating column can be obtained by assuming that the features are broadened by the relativistic Doppler effect.

For a mildly relativistic plasma the half width $\Delta\lambda_m$ of the mth harmonic is given by $\Delta\lambda_m/\lambda_m = m^{1/2}(T/(5.9\times10^9))$ (ref. 14). Using the observed value of $\Delta\lambda_7/\lambda_7$ 0.06, we find $T = 1.3\times10^8$ K. This temperature is consistent with the shock temperature expected for accretion onto a low mass white dwarf[12]. For a column of given temperature and thickness there is a critical value m^* of m above which the column becomes optically thin to cyclotron emission, and the intensity of the cyclotron radiation drops rapidly. The observed turnover of the

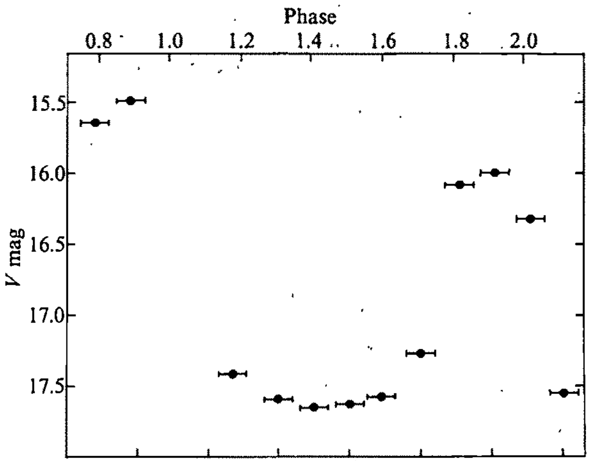

Fig. 1 Light variations for VV Pup during 2 February 1979 from 11.48 UT to 14.00 UT as determined from individual IDS scans with an effective time resolution of 8 min. Phase is given according to Walker's ephemeris.

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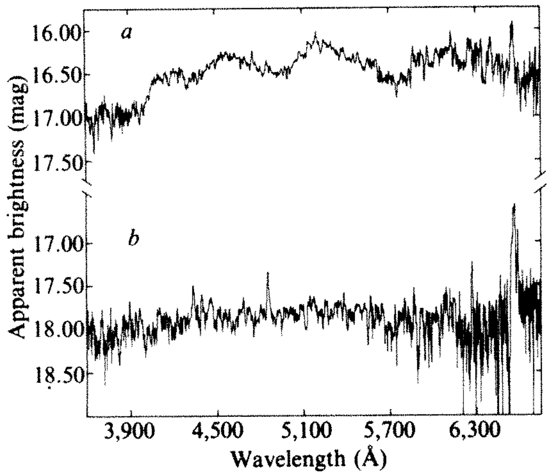

Fig. 2 IDS scans of VV Pup at 10 Å resolution on 2 February 1979 for *a*, the bright (11.48 UT to 11.58 UT) and *b*, the faint (12.27 UT to 13.10 UT) phases.

energy distribution at $\lambda \sim 4{,}100$ Å could then be interpreted as an optical depth effect.

Because X rays have hitherto not been detected in VV Puppis, there is no direct observational evidence, unlike AM Herculis, for the presence of a shock region. It is therefore possible that the features arise from a cooler region and that magnetic broadening effects are important. However, the eclipse data and luminosity criteria favour a high temperature ($\sim 10^8$ K) emitting region of small dimension[5,17].

The absorption features we have observed have not previously been detected in VV Pup. However, the long term variability of the light curve suggests that the height h of the optical column is variable. Values of $10^{-1}R$ and $10^{-2}R$ where R is the radius of the white dwarf, have been reported even at phases when the system had similar maximum brightness[5,6]. It is not clear how changes in the accretion rate are related to the height h, and width d, of the column. For a dipole field geometry and a cylindrical column at a pole a feature of half width $\Delta\lambda$ will survive magnetic broadening if $h/R \leqslant 1/3\ \Delta\lambda/\lambda$ and $d/R \leqslant 8/3\ \Delta\lambda/\lambda$. For the λ4,350 Å feature to be detectable we require $h/R < 0.02$ and $d/R \leqslant 0.16$. A large increase in h or d could easily result in the obliteration of the absorption features by magnetic broadening thus accounting for their non-detection by previous observers.

These spectra of VV Pup show hitherto unreported absorption features which are most probably high harmonic ($m = 6, 7, 8$) cyclotron absorption in the shock region near the surface of the white dwarf.

If this interpretation is correct, this would be the first determination of the magnetic field in the vicinity of a white dwarf in a close binary system. The analogy with Her X-1 where an X-ray cyclotron feature appropriate to the field of a neutron star has been detected is worth noting. Note also that the field strength deduced for VV Puppis lies in the range of field strengths deduced for isolated white dwarfs by the Zeeman effect[16,17].

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Optical counterpart of 2A0311−227

G. Williams, M. Johns, C. Price & A. Hiltner

Department of Astronomy, University of Michigan, Ann Arbor, Michigan 48109

F. Boley, S. Maker & D. Mook

Department of Physics and Astronomy, Dartmouth College, Hanover, New Haven 03755

The X-ray source 2A0311−227 was recently identified independently by Williams[1] and by Griffiths *et al.*[2]. Williams made a tentative identification from an inspection of low dispersion objective prism Schmidt plates taken at Cerro Tololo Inter-American Observatory for the discovery of active galaxies and quasars. The identifying characteristics were strong emission of H and He II. Griffiths *et al.* made the identification from an excellent HEAO-1 position followed by spectroscopic observations. The optical position of the object is α = 3 h 12 min 00.0 s, $\delta = -22° 46' 48''$ (1950). Figure 1 identifies the X-ray source among the neighbouring stars. It has a mag (variable) of about 15. Immediately after the tentative identification by Williams the 1.3-m telescope at McGraw-Hill Observatory on Kitt Peak was scheduled for spectrophotometric observations of the star in question, and we report the results here. A photon-counting spectral scanner was used. The instrument gives a dispersion of 1.15 Å per channel. Exposures of 5 and 10 min were made that covered the spectral region from 4,000 Å to 5,800 Å. A few scans were taken with lower dispersion and broader spectral coverage. The spectrum shows strong emission features, especially H I, He II λ4686 and the C III–N III blend at λ4640–4650 (ref. 2).

Analysis for radial velocities of the individual spectral scans obtained on the first night showed the star to be a spectroscopic binary of short period. Observations on subsequent nights established a period of 81.04 ± 0.01 min (ref. 1).

The velocity curve for Hβ is shown in Fig. 2. The phase was computed from HJD 2,443,915.6300 ± 0.0006 + 0.05628E. The semiamplitude is 355 km s^{-1}. The velocity curve for He II λ4686 is very similar. Any difference in either amplitude or phase is obscured by scatter in the observations. Both Hβ and He II λ4686 show a systemic velocity of +130 km s^{-1}. However, the projection of the solar motion at this galactic longitude and latitude ($l = 212.9$, $b = -57.4$) is 73 km s^{-1} which gives a galactic radial velocity of +57 km s^{-1}. The annual proper motion of $+0.147 \pm 0.013''$ and $-0.070 \pm 0.013''$ in right ascension and declination, respectively (W. J. Luyten, personal communication) indicates that the object is no more than a few hundred parsecs away.

The widths of emission lines are approximately 700 km s^{-1}. Their equivalent widths vary with phase. For example, Hβ changes by a factor of three. However, these equivalent widths must be corrected for variability of the underlying continuum. Preliminary data suggest that variations in the total flux will be small.

Blue and yellow light curves for this object were obtained at Carnegie Southern Observatory (data not shown). There are two maxima and two minima in visual light (5,300 Å); the two minima are of nearly equal depth, but the two maxima differ by 0.4 mag. The total variation is near 1.2 mag, at $V = 13.8$–15.0. A characteristic of the visual light curve seems to be a three-peaked structure at the primary maximum. The separation of the peaks is approximately 6 min and the drop in brightness between peaks is typically 0.3 mag. The central peak, which is also the brightest, occurs at phase 0.775. The maximum is in phase with the maximum velocity of approach. In the blue (4,300 Å) only one maximum is present, which again

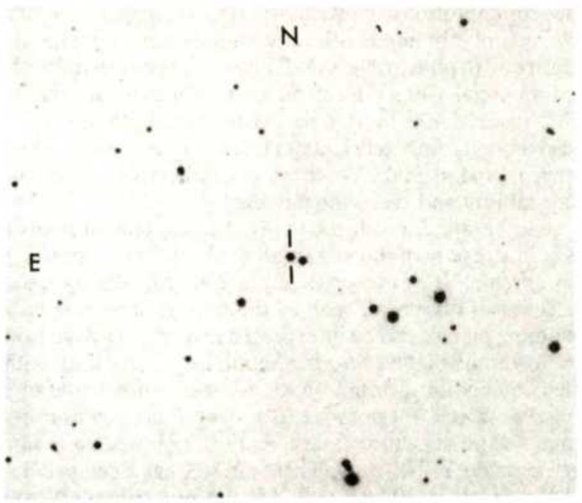

Fig. 1 Identification chart for 2A0311 − 227.

corresponds in phase to the primary maximum of the visual light curve. The total variation in the blue is about 0.6 mag. During one cycle of the blue light curve a large dip of 0.6 mag was observed during the maximum. This dip, which occurred at phase 0.712, had a width at half-minimum of about 2 min compared with 20–25 min for the maximum on which it occurred. One cycle later the dip was again present, although less pronounced. There is a colour variation, as one would expect from the differences in the blue and yellow light curves, with the star reddest at the two maxima of the yellow light curve.

This system has many characteristics in common with AM Herculis. The period is short (81 compared with 186 min), the spectral characteristics are similar (strong H and He II emission), the light curves for both systems are radically different in blue and yellow radiation[3], the two stars show considerable flickering[3] and both stars show variable circular and plane polarisation[4,5]. Each shows a short polarisation pulse that corresponds to maximum light[4,5]. In this system the polarisation pulse occurs at phase 0.742. Interestingly, this agrees very well with the dip in the visual light curve which precedes the central peak of the primary maximum. Finally, both objects have variable X-ray radiation[6,7].

The model for this X-ray source is therefore probably similar to that of AM Her—a white dwarf and a low mass main sequence companion[8]. We suggest a model with a white dwarf of 1.2 solar masses and a companion with 0.2 solar masses. This model follows from the work of Warner who has found that the masses of white dwarfs in cataclysmic variables are typically 1.2 solar masses[9]. He has also found that there is a relationship between the mass ratio and the orbital period. Using this relationship we find a mass of 0.2 solar masses for the secondary. In this model the separation of the two components is 0.7 $R_\odot$ and the main sequence component will just fill its Roche lobe. The collapsed star is most probably a white dwarf, as circular polarisation is observed[5]. Also, this model is compatible with the estimated distance from the proper motion.

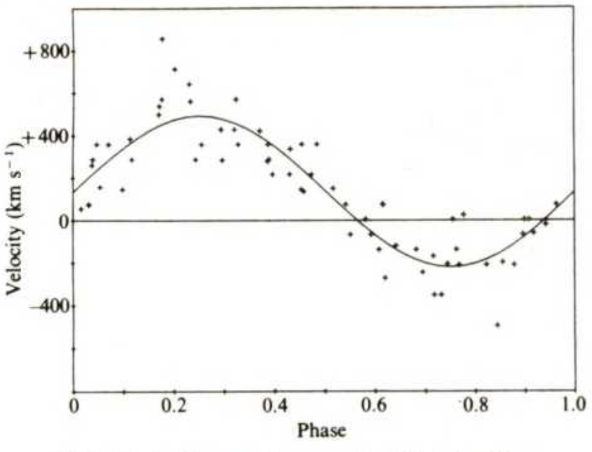

Fig. 2 The Hβ velocity curve for 2A0311 − 227.

The origin of the emission lines is unclear, but, as in other similar systems, they probably arise from the gas flow from the companion to the white dwarf. Further analysis of observational data may assist in establishing a more critical working model for this unique system.

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Atomic resolution with a 600-kV electron microscope

V. E. Cosslett, R. A. Camps*, W. O. Saxton & David J. Smith*†

Cavendish Laboratory, University of Cambridge

W. C. Nixon, H. Ahmed, C. J. D. Catto, J. R. A. Cleaver†, K. C. A. Smith, A. E. Timbs, P. W. Turner & P. M. Ross‡

Department of Engineering, University of Cambridge, UK

The resolution that may be achieved with an electron microscope can be improved by increasing the accelerating voltage, provided that the electrical and mechanical design is of a sufficiently high standard to realise the theoretical performance[1,2]. Several such microscopes have been constructed in recent years[3–5] and have produced images containing information which can be interpreted directly to a higher resolution than any yet obtained at 100 kV (refs 6, 7). The Cambridge University 600-kV high resolution electron microscope is now yielding high quality micrographs with directly interpretable resolution approaching 0.2 nm. We describe here some of the novel features of this microscope and give some measurements which establish its electron-optical performance. We also show some examples of recent observations which demonstrate the benefits of its atomic resolving power. A more detailed description of the instrument and more references are given elsewhere[2,8].

The electron gun incorporates a lanthanum hexaboride thermionic cathode, in the indirectly-heated rod configuration[9,10]. For operation with a brightness 10 times greater than is normally obtained from a tungsten filament the cathode lifetime is regularly more than 100 h. Such brightness allows observation of images on the fluorescent viewing screen at electron-optical magnifications > ×500,000, and hence permits direct and accurate correction of astigmatism and choice of objective lens defocus without recourse to lengthy trial and error procedures (compare Krivanek *et al.*[11]). Fringes of 0.27 nm spacing from a

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* Present address: High Resolution Electron Microscope, Department of Engineering, University of Cambridge, UK.

† To whom correspondence should be addressed.

‡ Deceased.

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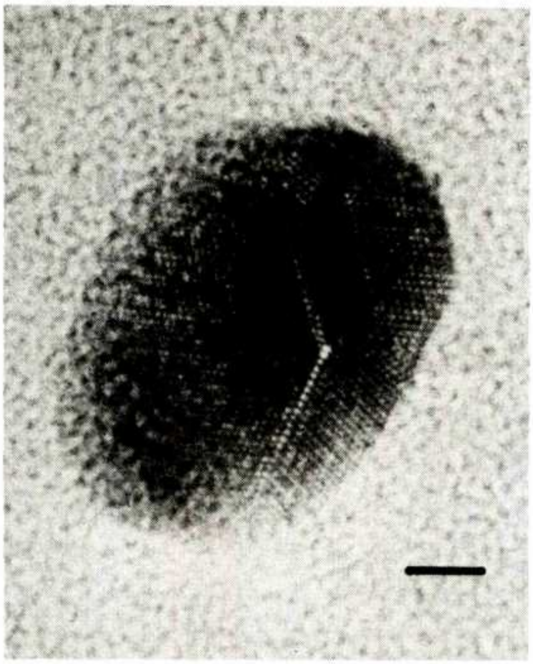

Fig. 1 Micrograph of a small gold particle, supported on an amorphous carbon film, recorded at 575 kV at the optimum defocus. Analysis of the detail in the carbon film defines the imaging conditions and indicates that structure within the particle can be interpreted directly as showing projections of rows of gold atoms separated by 0.235 nm, viewed end-on. Scale bar, 2 nm.

tungsten trioxide specimen have been observed on the fluorescent screen.

The objective lens of the microscope is a symmetrical single-field condenser-objective[12,13], with the specimen located close to the centre of the 14-mm polepiece gap. The calculated spherical aberration coefficient for this lens is 3.3 mm, so that for an accelerating voltage of 600 kV the contrast transfer function of the microscope should have a first zero crossing of 0.20 nm at the optimum defocus. This value provides a useful measure of the information which can be interpreted directly from a single micrograph, although details of finer spacing may be observable at this, and other, defocus values. To ensure that this performance is not degraded by electrical instabilities, highly stable lens current supplies have been developed and the 600-kV generator[14] has been modified to reduce ripple and drift.

The entire electron-optical assembly of the microscope, about 5.5 m high and weighing about 7 tonnes, is suspended from three compressed-air vibration isolation units mounted on the walls of the microscope room. Ambient vibrations have been shown to be attenuated by a factor of the order of 100, to a level at which there is no apparent deterioration of the image quality due to microscope vibrations. A side-loading specimen stage to fit within the polepiece gap (J. H. Lucas, Rickling, Essex) provides x and y tilts of ±30°, and some z motion. The rotational symmetry of optical diffractograms taken from micrographs of amorphous materials indicates that specimen drift is not generally limiting performance. High resolution operation can commence immediately after specimen exchange, which takes less than 3 minutes and uses a locally-produced airlock mechanism.

During the commissioning phase of the construction programme, most operation has been at an accelerating voltage of 500 kV. A recent micrograph of a thin film of amorphous carbon recorded at this operating voltage at the optimum defocus has been analysed using a small local computer system[15]. This analysis showed continuous information transfer, without oscillations in phase, to a resolution of 0.22 nm, indicating that the microscope is indeed capable of providing image details of atomic dimensions. Micrographs can then be interpreted directly to this resolution without recourse to lengthy calculation and prior knowledge of the specimen structure. Details of atomic configurations, particularly in the region of crystal defects, can be obtained without complications in image interpretation due to phase reversals. This is to be contrasted with the best commercial 100 kV electron microscopes where the interpretable resolution is limited to greater than 0.28 nm (ref. 16). Lattice fringes as fine as 0.102 nm have also been recorded with this microscope at 500 kV—these are indicative of the instrumental stability and resolving power.

The micrograph reproduced in Fig. 1 is one of a through-focal series of images which shows a small gold particle supported on a thin carbon film. It was recorded at the optimum defocus, so that image features observed, such as the configurations at each of the twinning planes, can be interpreted in terms of projections of atomic columns. However, it should be noted that without detailed calculations, based on an accurate knowledge of particle thickness, it is not possible to ascribe exact positions to the columns[17]. The structure image in Fig. 2 shows the complex inorganic oxide $Ti_2Nb_{10}O_{29}$. This material has been previously studied in detail at 100 kV (ref. 18) but not apparently at any higher voltage, although the isostructural $Nb_{12}O_{29}$ has been observed at high resolution at an accelerating voltage of 1 MV (ref. 19). The finest specimen features observed at 100 kV in $Ti_2Nb_{10}O_{29}$ which can be interpreted directly in terms of its known structure are the cation positions at the centre of the corner-shared metal–oxygen octahedra which are separated by 0.38 nm. The present image shows much finer interpretable detail, in particular resolving the cations in the edge-sharing octahedra along the regularly-spaced crystallographic shear planes. In a b-axis projection, the centres of these octahedra are separated by only 0.19 nm.

These initial results, and the measured contrast transfer function, indicate that this 600-kV electron microscope has an atomic resolving power, and can be expected to yield substantial and significant information about structures and defects at the

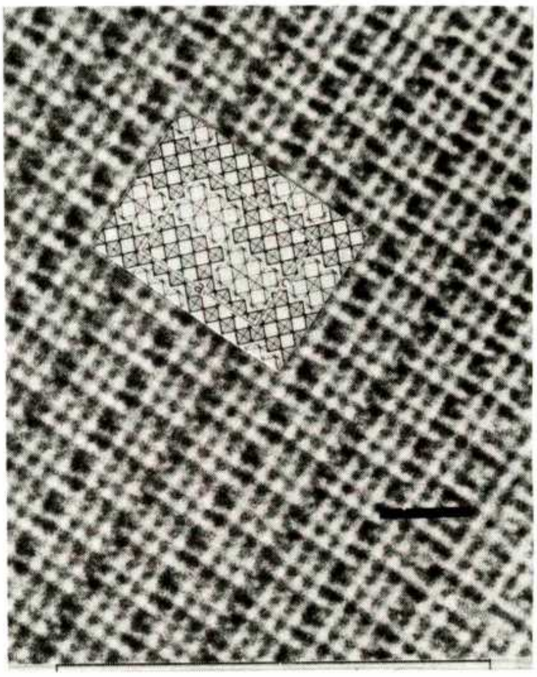

Fig. 2 A structure projection image of the complex inorganic oxide $Ti_2Nb_{10}O_{29}$ in a b-axis projection, recorded at 500 kV at the optimum defocus. As shown in the insert model, this material consists of 3 × 4 blocks of corner-sharing metal–oxygen octahedra, with the central cations separated by 0.38 nm. These blocks, which are at two levels within the unit cell, are joined by edge-sharing octahedra along the regular crystallographic shear planes, with octahedral centres separated by 0.19 nm in this projection. Near the crystal edge, the micrograph can be interpreted directly in terms of this structure although, in thicker regions, a slight misalignment of the crystal is indicated by lack of mirror symmetry. Scale bar, 2 nm.

atomic level for a wide range of amorphous and crystalline materials, covering many scientific disciplines. Applications have already commenced; there is good agreement between calculated and observed images of silicate minerals[20]. Other materials under investigation include amorphous metals, multiply-twinned particles, and intercalated graphite catalysts.

The Cambridge University 600-kV high-resolution electron microscope has been constructed with major financial assistance from the SRC. We thank R. H. Pryor and his staff for their assistance, Drs P. W. Hawkes, M. E. Haine, W. D. Riecke and Mr D. A. S. Rose, and many others, who have contributed to the success of the project, and Dr S. Iijima for providing the $Ti_2Nb_{10}O_{29}$ specimen.

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Individual silicate chains in wollastonite by high resolution electron microscopy

D. A. Jefferson & J. M. Thomas*

Department of Physical Chemistry, University of Cambridge. Lensfield Road, Cambridge

D. J. Smith & R. A. Camps

Department of Physics, University of Cambridge, Old Cavendish Laboratory, Free School Lane, Cambridge

C. J. D. Catto & J. R. A. Cleaver

Department of Engineering, University of Cambridge, Trumpington Road, Cambridge UK

Chemists and mineralogists often have to elucidate the crystal structure of new materials even when conventional X-ray methods are not applicable. If, for example, long-range order is not extensive or the amount of material available for examination is minute ($<10^{-7}$ g) the classical techniques of X-ray or neutron diffraction cannot be used. In such circumstances, direct structural determination using high resolution electron microscopy is, in principle, possible. To achieve worthwhile information, however, a point-to-point resolution of 2.8 Å or better is desirable, if the structural details of most oxides, halides, chalcogenides and various oxysalts of metallic and semi-metallic elements are to be established. We demonstrate here how high voltage, high resolution electron microscopy confirms the structure of a representative silicate mineral, showing the potential of the technique for the elucidation of locally abnormal (at the subunit cell level) structures.

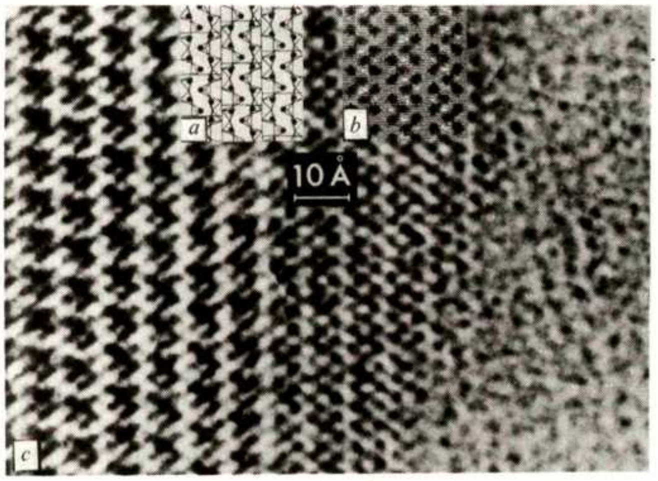

Fig. 1 *a*, Projection down *z*-axis of the idealised structure of wollastonite showing disposition of corner-sharing tetrahedra and Ca^{2+} ions (filled circles). *b*, Computer simulated image (see text and ref. 8). *c*, High resolution micrograph, taken in conditions specified in text.

Currently available 100 kV commercial electron microscopes, fitted with conventional (30°) goniometer stages, cannot structurally resolve (that is, form many-beam images in which each beam has the correct relative phase with respect to the others) interatomic distances of less than ~3.2 Å. (It is, of course, possible to obtain lattice fringes separated by less than 1 Å, but these carry only partial structural information.) Consequently most solid-state chemical work carried out using electron microscopy has until now involved structures based on the linkage of corner-sharing octahedral coordination groups, in which the ion exhibiting the sixfold coordination (for example W or Nb) is largely responsible for the scattering contrast seen in the projected image (of a sufficiently thin specimen). The 'block' and 'shear' structures, where the separation distances of the heavy cations are >3.5 Å are good examples[1–6].

Silicate minerals, which encompass a wide diversity of topologies that arise from various ways of linking SiO_4 tetrahedra, constitute important tests for the methodology of high resolution electron microscopy, principally because there are good reasons for suspecting that hitherto unknown structures can be revealed by direct, real-space examination of minute regions (encompassing some 10^4–10^6 units cells) of naturally occurring materials and their synthetic analogues. Indeed, new topologies (such as multiple-chain structures related to the amphiboles) have recently been identified[7,8] by comparing computed and observed images of ultrathin specimens in various electron-optical conditions. Furthermore, silicates serve as stringent test specimens for high resolution microscopy because: the central tetrahedrally coordinated atom (Si or Al) displays little inherent contrast in comparison to its surrounding atoms, and the inter-silicon distances are significantly smaller (~2.8 Å) than comparable previously imaged distances. Also, the spaces between anionic chains in the silicate network are occupied by cations such as Na^+ and Ca^{2+}, which have scattering powers comparable with those of the atoms in the silicate (or aluminosilicate) macro-anions. Therefore, the interpretation of image contrast is less straightforward and certainly less reliable on an intuitive basis than the contrast displayed by the 'shear' and 'block' structures.

Observations of the pyroxenoid mineral, wollastonite ($CaSiO_3$) have been made with the Cambridge University 600 kV high resolution transmission microscope[9,10]. Micrographs demonstrating its atomic resolving power are shown in the preceding paper[11].

The initial results closely match previously computed[8] images for wollastonite. The structure, as deduced from X-ray work[12] on ordered specimens, consists of infinite one-dimensional chains of corner-sharing SiO_4 tetrahedra (Fig. 1*a*). Si–Si distances within the chains are 3.01 and 3.15 Å, but in projection down the *z*-axis, these reduce to 2.71 and 3.01 Å. Note that in this projection the Ca^{2+} ions which link adjacent chains more or less coincide with the centres of the SiO_4 tetrahedra, and have a separation of 2.9 Å. Computer simulations indicate that a structural resolution of better than 2 Å is required to reveal pairs of chains and the clearly identifiable calcium ions. The computer-simulated image[8] (Fig. 1*b*), in the same projection as Fig. 1*a*, was calculated using the thin phase grating approximation (Δz = 10 Å) with instrumental parameters: accelerating voltage, 600 kV; coefficient of spherical aberration (C_s) 3.3 mm; defocus of 730 Å; and giving a first zero on the phase contrast transfer function of 1.81 Å. The fine structure of the silicate chains is clearly resolved in this simulation, groups of three SiO_4 tetrahedra, and their attendant Ca^{2+} ions, being readily visible.

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* To whom correspondence should be addressed.

Results obtained with an actual specimen of wollastonite in the Cambridge microscope can now be compared with the computer simulation. From optical diffraction studies[13,14], we estimate that information in micrographs such as Fig. 1*c* extends to 1.44 Å at the edges of the crystal, where the thin phase grating approximation is observed to hold. This micrograph is from a through-focal series of a wedge-shaped crystal of wollastonite, taken with an accelerating voltage of 500 kV with $C_s \sim 3.5$ mm. The phase grating approximation was observed to be valid within a 50–60 Å region at the periphery of the crystal and within this area the observed arrangement of SiO_4 tetrahedra closely resembles that of the computer simulation.

These results show that the prospects of directly investigating the ultrastructure of a wide range of partially ordered minerals and inorganic solids[2,8,15,16] (borates, phosphates and other classes mentioned earlier) are very encouraging. Such studies of a range of silicates are now in progress.

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A projected potential image of a defect in an ordered alloy?

W. M. Stobbs

Department of Metallurgy & Materials Science, Pembroke St,
Cambridge, UK

R. Portier

CECM Laboratoire propre du CNRS, 15 rue Georges Urbain,
94400-Vitry/Seine, France

The high-resolution performance of 100-kV transmission electron microscopes has improved dramatically in the past few years. Use of an objective lens with a spherial aberration coefficient C_s of 0.7 mm and a low incident beam divergence ($\alpha = 0.2$ mrad) allows point-to-point resolutions shown by Bursill and Wood[1] to be ~1.6 Å. These authors, however, distinguish between this 'instrumental' resolution and the resolution which can in principle be used for direct structural resolution and interpretation—only slightly better than 3 Å in the above conditions. The function sin χ, the part of the lens transfer function most directly dependent on the phase changes χ of the electrons as a function of lens defocus and spherical aberration, shows rapid oscillations for spacings smaller than ~2.8 Å even for the optimum defocus condition for which sin χ is apparently −1 over a maximum range of spacings[1]. Thus even if point-to-point resolutions in the 2–3 Å range are obtained, by careful limitation of the beam divergence, the images can only be related to the structure by very careful comparison with computed images. It is this fact and the limited improvement to be expected on any further reduction of C_s, as well as the severe geometric limitations imposed on specimen manipulation in such lenses, which has lead to the approach of using increased accelerating voltages with a concomitant reduction in electron wavelength, λ: the above 'structure resolution' limit is approximately $0.7C_s^{1/4}\lambda^{3/4}$. Most of the high-resolution work to date has concentrated on the examination of materials in which there are strong projected charge distribution periodicities at relatively coarse spacings. To obtain further information on the difficulties involved in the examination of defects in metals (with generally smaller spacings of the important charge projections) we have studied the degree to which images of the ordered alloy Ag_3Mg can be related to naive descriptions of the projected charge distribution.

The Nb_2O_5–WO_3 system is one in which relatively coarse spacings arise. Iijima[2] has obtained particularly beautiful micrographs of this system which are clearly directly interpretable. Other work has been done on silicon and germanium and very good apparent resolutions were obtained by Izui *et al.*[3] on silicon. While the images are very similar to those naively expected for atomic resolution at 1.36 Å along [$\bar{1}$00] in the [110] projection the work of Spence *et al.*[4] and Bursill and Wood[1] suggests that the real instrumental resolution was closer to 1.6 Å, the figure arrived at by the latter authors in their examination of Ti_6O_{11}. This oxide is triclinic and while in the [1$\bar{1}$1] rutile projection discussed by Burstill and Wood[1] the Ti atom column spacing is generally 2.96 Å, near the crystallographic shear planes (CSP) which the material contains at 9.6 Å spacing the projected separation is considerably less. The experimental and computed images are thus of particular interest as the image contrast is complex and shows rapid variation with defocus in a way which is more characteristic of the behaviour to be expected near defects in metals where the symmetry of the local projected potential can be similarly complex.

The minimum spacing of the columns of Mg atoms in the ordered alloy Ag_3Mg (when viewed in the 010 direction (Fig. 1)), is 4.0 Å and thus, given the difference in atomic number

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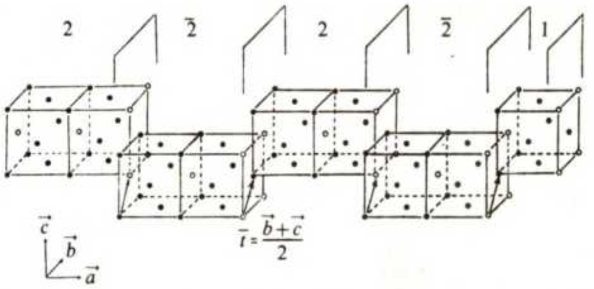

Fig. 1 Diagram, showing the displacements associated with a $(2\bar{2}2\bar{2}1)_2$ long period structure with a repeat in the *a* direction of 72 Å. ●, Ag; ○, Mg.

between Ag and Mg, images showing the characteristics to be expected for the projected charge distribution can be obtained with relative ease. The material also contains periodic antiphase boundaries (PAPB) which have been shown to be strictly planar with 100 normal[5], the translation being $(b+c)/2$. The spacings of the important charge projections at these boundaries as viewed in the 010 direction thus provide an interesting test situation for the appearance of high resolution images. As a function of the degree of stoichiometry the alloy also shows a long period structure apparently controlled, at least over a range of composition, by the atomic ratios[5] with regular insertion of single-cell displacements in a repeat which otherwise shows PAPB every two cells. A diagram of an example of such a structure described, on Fujiwara's[6] notation, as $(2\bar{2}2\bar{2}1)_2$ with a repeat in the *a* direction of 72 Å is shown in Fig. 1. Specimens with structures of this general type show occasional imperfections in the long-period arrays in which the apparent position of the single cell part of the array is displaced (for example, $2\bar{2}1\bar{2}2 \rightarrow 2\bar{2}2\bar{1}2$). In an analysis of the way in which the structure itself arises it is of particular interest to determine whether defects of this type are purely displacive or associated as well with progressive changes in the Mg-to-Ag ratios in the relevant columns. Can this question be answered by examination of images showing characteristics away from the defect as might be expected, on a naive interpretation, in terms of the projected charge density?

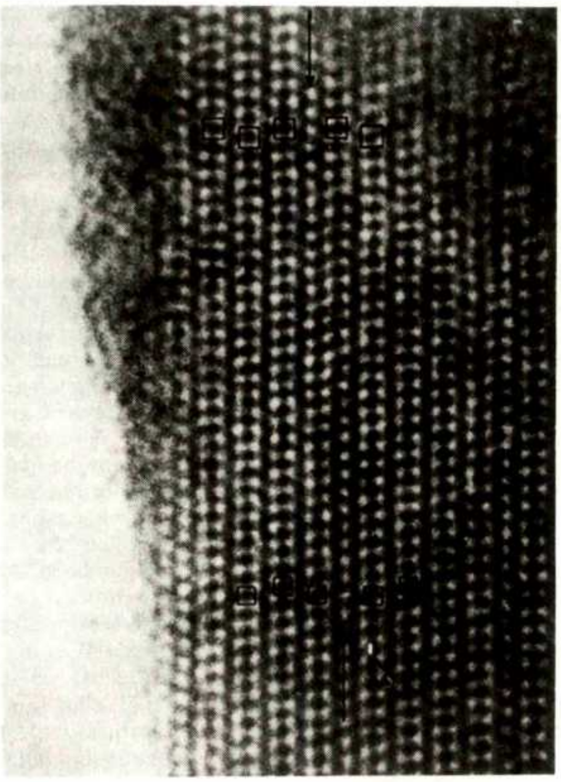

Fig. 2 Axial electron micrograph of a thin region where the position of the single cell width is changed in the long-period structure. The structure is as shown in Fig. 1.

An example of an image of such a defect in the ordered structure is shown in Fig. 2. The micrograph formed part of an extended through focal series. The series was obtained in axial conditions, the specimen being accurately oriented at 010 in a top-entry JEOL tilting holder, in a JEOL 100CX microscope, though with the specimen in a slightly lowered position where Cs is approximately 2.5 mm rather than 3.3 mm as it is for the normally used position in the relatively low resolution pole-pieces of the standard objective lens of this microscope. While a pointed filament was used with a nominal beam current of less than 10 μA and a gun bias sufficient to prevent space charge energy spread limitations in the gun, the images were obtained using overfocussed illumination with $\alpha \simeq 0.5$ m rad. This figure is high and for optimum instrument resolution α should normally be limited to a value less than half this figure: as is well known[7] the envelope function for $\sin \chi$ is controlled not only by the chromatic aberration but also by a combination of the chromatic and spherical aberrations with the convergence and under normal circumstances it is the convergence which is most important.

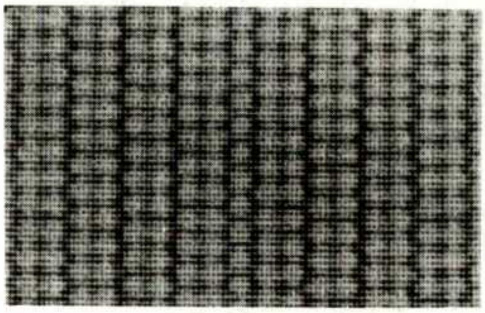

Fig. 3 Image simulation for the model structure shown in Fig. 1 under the experimental conditions used to obtain the micrograph in Fig. 2. C_s, 2.6 mm; Δ_f, 3,040 Å; specimen thickness, 80 Å. A polynomial expansion technique was used and further details of this and other comparative simulations for a variety of thicknesses will be published elsewhere[8].

At first sight the image (Fig. 2) shows features, at least away from the defect, which might be interpreted directly in terms of the projected charge density: the regular $(2\bar{2}2\bar{2}1)_2$ form of the ordering may be clearly visualised. Furthermore in the relevant region the through focal series showed only images of this general type, or ones showing minimal contrast. Further back in the foil a variety of features were observed showing rapid variation with defocus. In fact two other images in the series showed the features characteristic of Fig. 2, both with reversed contrast but with minor differences. Image calculations have been completed which match the observed changes in contrast and an example of such a computation appropriate for comparison with Fig. 2 is presented in Fig. 3. While these calculations will be discussed in more detail elsewhere[8], the simulations and optical diffraction patterns obtained from the contamination at the edge of the foil showed that the three relevant images corresponded to defocus situations at which the 4 Å spacing was optically transferred in the 3rd, 4th and 5th reversals of $\sin \chi$ respectively. The calculations, for an arbitrary but not unreasonable thickness of 80 Å, also demonstrated, however, that the amplitude and phase changes of the various diffracted beams associated with the periodicity of the ordering should have been sufficiently high to make any application of a concept based on the weak phase approximation totally inappropriate. We thus have the intriguing situation that the gross 4 Å periodicity, characteristic of the projected charge distribution, is readily visualised partially because of the relatively high beam convergence reducing the amplitude of the transfer function for the lower spacings readily seen in thicker foils.

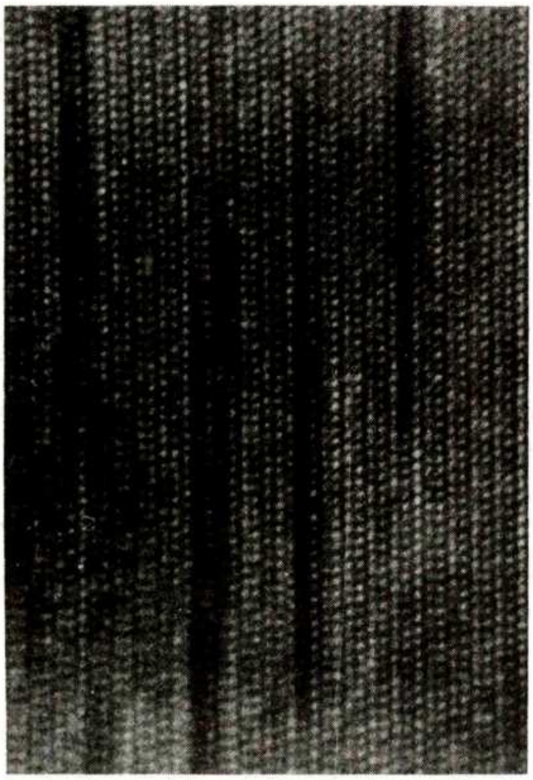

Fig. 4 Axial electron micrograph of a relatively thick region (compare with Fig. 2) at a defocus at which the structure may be visualised. The strong contrast at the defects at this thickness is a clear indication that application of the weak phase approximation would be inappropriate.

Accordingly, although the image in the region of the defect shows none of the features associated with strong diffraction, as are seen in thicker foils (Fig. 4) (see for example ref. 9) and which would normally warn one against any direct interpretation, it is clear that the regular displacements of the apparent projected charge columns near the defect have no simple interpretation. Furthermore the interpretation given here suggests that even with an objective lens with $C_s = 0.7$ mm and low beam convergence no simple interpretation would be possible at the defect not only because the relevant spacings (2 Å) would still lie beyond the first zero in the transfer function but also, and as importantly, because the amplitude and phase changes of the diffracted beams would be too high for the weak phase approximation to be relevant. We have thus demonstrated that in perfect crystals the use of a moderate beam convergence coupled with careful tuning of the transfer function allows images to be produced which, given comparison with computation, bear simple relations to the projected potential despite the main features of the image being transferred well beyond the first zero in the transfer function. That this is so does not, however, allow such an interpretation of a defect and the fundamental question of whether or not the defect is essentially displacive or diffusional will probably be answered only with the advent of a direct structural resolution of 2 Å as may be possible at higher kilovoltages.

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High resolution imaging of amorphous materials

W. M. Stobbs

Department of Metallurgy and Materials Science, University of Cambridge, Pembroke Street, Cambridge, UK

David J. Smith

High Resolution Electron Microscope, Department of Engineering, University of Cambridge, Free School Lane, Cambridge, UK

The important role of electron microscopy in the study of amorphous materials is in the examination of the local variation in structure, whether inherent or caused by deformation, irradiation or the nucleation of crystallisation. This requires that the image contain information about local spacings and their variability at the atomic level even if only in relation to a projection. Until recently commercial instruments could not do this for spacings less than about 0.3 nm, and material scientists have either concentrated on the interpretation of coarser structural irregularities in amorphous alloys or used indirect methods to get round the lack of resolving power of the objective lens of their microscopes (see ref. 1 for review). The interpretative dangers of the most obvious approach of using tilted illumination conditions are now well understood[2–5]. While the prospects for dark field interference techniques are a little more promising[6] the essential problem of a nonlinear transfer function remains[7]. More interesting, though with other limitations, is the use of hollow cone illumination[8] with axial transfer[8–11]. Here we present high resolution, axial illumination electron micrographs of such materials with detail at spacings less than 0.2 nm and discuss the difficulties involved in the interpretation of such images.

A JEOL 120CX with a strong objective lens ($C_s = 0.7$ mm) was used to obtain images of amorphous materials in the axial mode showing detail down to 0.17 nm and thus potentially capable of interpretation at the atomic level. This might initially suggest that microscopists should be able to tackle the types of problem mentioned above immediately. However in order to understand how images of, for example, deformation bands may be interpreted it is essential to examine first the extent to which detailed information can be gained on atomic coordination in an 'averagely uniform' amorphous material. Thus, after describing our results, we discuss the extent to which this may be done for the technique used.

The technique of tuning the defocus to show up particular spacings in the image is well known for crystalline objects as is the method of choosing a defocus such that the minimum of the phase change covers a broad band of spacings of interest giving them all phase changes approximating to $(2n+1)\pi/2$. Shifting the 'broad band' transfer interval, in this manner, as the spacing examined gets smaller, becomes increasingly important, particularly because it is here that the transfer is least affected by the beam convergence. We have essentially applied the same arguments to the study of amorphous materials and show that, provided the image resolution is not limited by chromatic aberration, the broad band transfer may be tuned to the spacings of interest for a given material down to spacings of ~0.2 nm.

As examples of images of amorphous materials we show micrographs of phosphorus (Fig. 1) and an FeNiB alloy (Fig. 2). The former image was obtained at 120 kV with a defocus of ~170 nm; the latter, at 100 kV, with a defocus of ~250 nm. The convergence angle of the incident illumination, as suggested by the central spot in the electron diffraction pattern was less than 10^{-3} radians, and a pointed filament was used at a beam current of approximately $8\,\mu$A. The images were recorded at magnifications of approximately 500,000 as calibrated using graphitised carbon test specimens with an error of less than 5%. The optical diffraction pattern of the 120 kV calibration image shown in Fig. 1*b* may be compared directly with that from the phosphorous image in Fig. 1*a*.

It is clear that, in the specified conditions, the optical diffraction pattern of the FeNiB alloy demonstrates transfer down to ~0.2 nm in the amorphous region. The spots in the diffraction pattern (Fig. 2 inset) are associated with the patch of regularly spaced fringes in this particular image area which are at a spacing of approximately 0.19 nm. It is tempting to suggest that the alloy contained some microcrystalline regions in an amorphous sea, or at least increased order, but it also possible that we are seeing the first stages of crystallisation under the beam. The number density of such regions in the electron irradiated area is, however, small and this particular patch was present when the first micrograph was taken which was within 30 s of irradiation. Since crystallisation at the 1.0–2.0 nm level is normally only visible in the conditions used after about 10 min, it is possible that it was present before irradiation. The point of interest is, however, that the first ring in the electron diffraction pattern of this amorphous alloy is centred at a radius of about 5 nm^{-1}. Thus, taking the optical diffraction pattern to be a fair indication, the image of the amorphous region ought to contain information about the local atomic arrangements or at least their projection at this level of resolution. Since the electron diffraction pattern of amorphous phosphorus indicates that there is strong scattering associated with spacings around 0.5–0.6 nm and 0.23 nm the image in this case ought to be even more easily interpreted: at the higher kilovoltage the optical diffraction pattern (Fig. 1*b*) indicates transfer to 0.17 nm.

We must now, however, ask whether or not the optical diffraction pattern is a fair guide to the point to point interpretable information in the micrograph. Consider the appearance of optical diffractograms of axial lattice images. If the crystal is finite, subsidiary maxima appear at 2 g whether or not these fringes are present in the image. Again, even if these fringes are present they can arise through the interference of phase correlated scattering between +g and −g Bragg reflections and thus may not be indicative of transfer at the angles corresponding to 2 g. Lattice fringes with 0.1 nm spacing at the 001 normal of a gold crystal can be obtained axially but this is indicative of transfer corresponding to spacings of 0.2 nm. In the same material the 0.14 nm 220 lattice fringes may be obtained axially at positions away from the normal where only this systematic row of reflections is strongly excited. This does indicate transfer at this inverse spacing. It is, however, well known that this is much easier than demonstrating equivalent transfer for an amorphous material because relatively larger regions scatter coherently in a crystal than in an amorphous specimen.

The most worrying aspect of the points raised above is that the spacings imaged for the amorphous material, as indicated by the optical diffraction pattern, could result from the equivalent of +g: −g interferences as described for a crystal. However, the phosphorous used was <10 nm thick and this explanation does not seem likely. That locally etched regions appear to show structure at lower visibility than elsewhere can also be explained on a solely first order scattering approach. We are comparing transfer function behaviour for a variety of materials as a function of their thickness to get more information on this point, as well as on the extent and lack of zeros in the optical diffraction pattern at the smaller spacings as a function of the convergence employed.

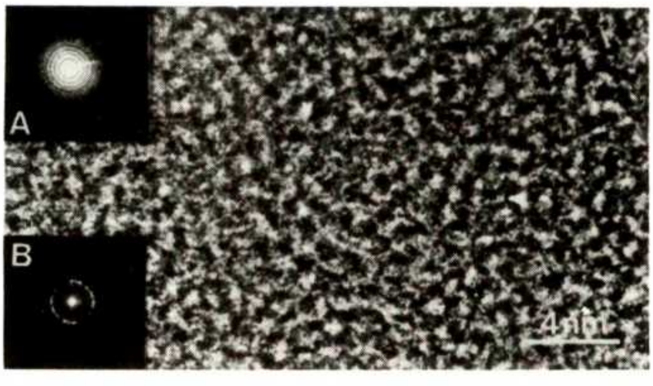

Fig. 1 Image of phosphorus obtained axially at 120 kV. Defocus ~170 nm. Insert *a* is an optical diffractogram showing transfer to approximately 0.17 nm. Insert *b* is from graphitised carbon on the same scale and shows transfer at 0.34, 0.21 and 0.17 nm.

Fig. 2 Image of an FeNiB alloy obtained axially at 100 kV. Defocus ~250 nm. The optical diffractogram shows the transfer of the locally crystalline region at 0.19 nm (the scale is different from Fig. 1).

With the provisos mentioned above, the results described suggest that suitable analysis of a through focal series of images with the deconvolution of the effect of the transfer function should be possible[12], at least down to spacings at which the ring structure of the transfer function may be seen in the optical diffractograms (~0.2 nm). While recording the images requires some care in using a relatively low beam current, the low C_c of the objective lens of the JEOL 120CX means that under normal circumstances the most important experimental aspects are to keep a low beam convergence and to tune the broad band of the transfer function to the spacings of interest.

Note that images of the type described here, where the relevant resolution is attained at relatively large values of defocus, and concommitantly chopped transfer, require considerable computational analysis before a full projection of the amorphous structure may be visualised. (It is extremely difficult to do this without recourse to the weak phase approximation and it is becoming increasingly obvious[13,14] that this is not very realistic even for the extremely thin specimens normally examined (<10 nm) unless they are of low atomic number.) If the weak phase approximation is not valid then the computation of the relation between the projected structure and the image is much easier if there is continuous transfer out to the lower spacings of interest for the given specimen. This can be done most easily by increasing the accelerating voltage.

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Unsaturated water flow within porous materials observed by NMR imaging

R. J. Gummerson, C. Hall & W. D. Hoff

Department of Building, University of Manchester Institute of Science and Technology, PO Box 88, Manchester, UK

R. Hawkes, G. N. Holland & W. S. Moore

Department of Physics, University of Nottingham, University Park, Nottingham, UK

Wetting, drying and permeation processes in various porous materials are of special interest to soil science and agriculture, building science and chemical engineering. Direct experimental study of unsaturated flow within such materials is hampered by the difficulty of detecting with precision changes in their internal water content distributions. In laboratory work on soils gravimetric sampling is widely used[1]. However, this is a destructive method which interferes with water flow processes and in any case is not easy to apply accurately to rigid materials such as permeable rocks, ceramics and building materials. γ-ray attenuation is the only established non-destructive instrumental method in laboratory use[2,3]. We report the first use of a nuclear magnetic resonance (NMR) imaging technique to monitor the dynamics of the internal water content distribution in several porous inorganic materials during capillary inflow.

The NMR imaging technique allows NMR signals to be isolated selectively from individual volume elements within a structure containing NMR-active nuclei. The major aim of work on this technique is to develop an imaging method for use in medical diagnosis, complementary to existing methods such as X-ray tomography. A description of our method of NMR image formation and examples of its medical use have been given elsewhere[4–6]. However, the technique is applicable to the study of the distribution of nuclear density within any non-metallic material. In the present case, the hydrogen nuclei (protons) of mobile water molecules within the pores of the material give rise to the NMR signal of amplitude proportional to the water content in defined volume elements.

Our apparatus uses an iron-cored electromagnet generating a field of 0.7 T, corresponding to a proton resonance frequency of 30 MHz. The magnet has a horizontal cylindrical sample compartment of diameter 80 mm and length 600 mm. This configuration is well suited to the study of the movement of the wetting profile during horizontal capillary absorption of water into long rectangular specimens with sealed sides (approximating to one-dimensional absorption into a semi-infinite solid). Subsequently redistribution and drying processes may be observed.

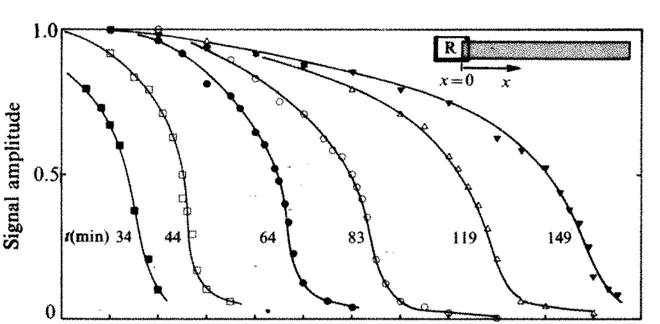

Fig. 1 Water content profiles obtained by NMR during capillary absorption of water by a plaster bar. Elapsed times t are shown. The bar (235 × 33 × 33 mm) was cast from a mix (1 : 2 by volume) of a commercial hydrated lime and a commercial retarded hemihydrate plaster (class B, type b2 gypsum building plaster). After setting and drying, all side faces were sealed with an epoxy coating. Inset sketch shows bar with attached reservoir R to supply water to the inflow face during the experiment. Signal amplitude is scaled to the range 0–1, corresponding to a reduced water content (θ) range of 0–1. $u_1 \sim 0.45$ cm^3 water per cm^3 dry solid.

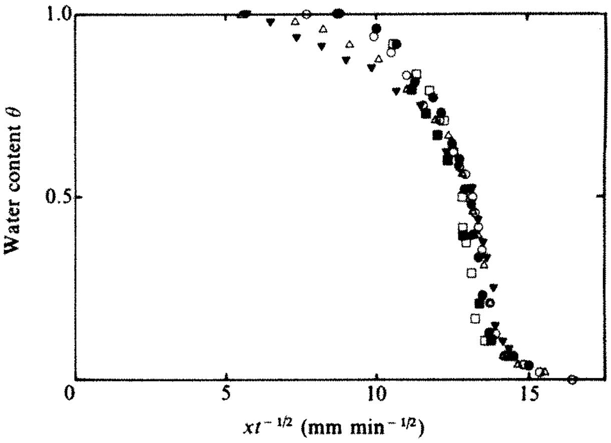

Fig. 2 Water content profiles of Fig. 1 replotted against $xt^{-1/2}$ ($= \phi$) to form a θ–ϕ master curve.

The NMR signal was collected from a magnetically defined thin slice of the specimen transverse to the direction of flow. The complete water content profile was obtained point by point by stepping the specimen in the direction x through the defined slice, enabling the water content in the slice to be determined at a number of distances from the inflow face ($x = 0$). The time needed to measure the NMR signal amplitude from each slice was about 10 s; thus a complete profile could be obtained in about 5 min. Figure 1 shows a family of water content profiles determined during capillary absorption into a gypsum-lime plaster bar. The signal amplitude is scaled to correspond to the range 0–1 in θ, the reduced water content. ($\theta = (u - u_0)/(u_1 - u_0)$ where u is the volumetric or gravimetric water content and subscripts 1 and 0 indicate nominal saturated and dry states[7]. All materials used were initially air-dry; thus $u_0 \approx 0$ and $\theta = u/u_1$.) The shape of the leading portion of the profile (at the lowest water contents) is sensitive to the defined slice thickness. Subsequent measurements showed a steeper intersection with the x axis (see Fig. 3), and a resolution of <2 mm.

Much of the analysis of unsaturated flow developed in soil physics[8] and more recently applied to water transport in building materials[7,9] is based on the extended Darcy equation[10]. If valid, this means that one-dimensional horizontal flow is described by

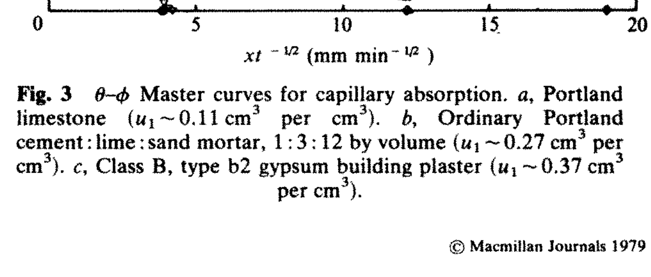

Fig. 3 θ–ϕ Master curves for capillary absorption. *a*, Portland limestone ($u_1 \sim 0.11$ cm^3 per cm^3). *b*, Ordinary Portland cement : lime : sand mortar, 1 : 3 : 12 by volume ($u_1 \sim 0.27$ cm^3 per cm^3). *c*, Class B, type b2 gypsum building plaster ($u_1 \sim 0.37$ cm^3 per cm^3).

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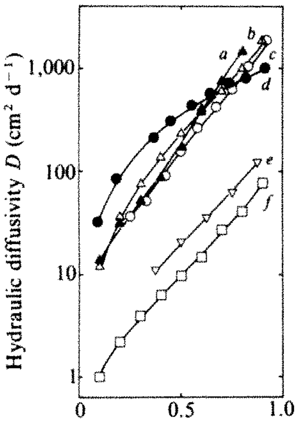

Fig. 4 Hydraulic diffusivity $D(\theta)$ of six materials calculated from NMR capillary absorption profiles. *a*, Cement : lime : sand mortar (1 : 3 : 12). *b*, Gypsum building plaster. *c*, Gypsum building plaster + hydrated lime. *d*, Locharbriggs sandstone ($u_1 \sim 0.25$ cm^3 per cm^3). *e*, Corsehill sandstone ($u_1 \sim 0.24$ cm^3 per cm^3). *f*, Portland limestone.

the nonlinear diffusion equation

$$\frac{\partial\theta}{\partial t}=\frac{\partial}{\partial x}\left\{D(\theta)\frac{\partial\theta}{\partial x}\right\}$$

where θ is the water content and D is the unsaturated hydraulic diffusivity, a property of the porous material. This equation has the solution $x(\theta, t) = \phi(\theta)t^{1/2}$ for absorption at a wet face ($\theta = 1$) into an initially dry ($\theta = 0$) semi-infinite homogeneous material. Thus the water-content distance profile advances as $t^{1/2}$, maintaining constant shape $\phi(\theta)$. Figure 2 shows that the profiles of Fig. 1 when replotted against $xt^{-1/2}$ fall approximately on a single θ–ϕ master curve. Figure 3 shows similar master curves obtained from capillary inflow profiles on three other materials.

These results, taken together with the reworking (unpublished) of two sets of γ-ray data[11,12] on autoclaved aerated concretes and with a number of studies on soils[13] provide strong support for the applicability of the extended Darcy equation to such porous materials. From θ–ϕ data the unsaturated diffusivity $D(\theta)$ may be calculated for each material by the method of Bruce and Klute[14]. Results for six materials are shown in Fig. 4.

Several other wetted porous materials have also been examined. Apparently satisfactory signals were obtained from a clay loam, and from two sand samples. The NMR response from several specimens of fired-clay building bricks and from an autoclaved aerated concrete was extremely weak, indicating an exceptionally short nuclear relaxation time. A calcitic sandstone also gave a very weak response. The factors controlling signal amplitude from different materials have not been investigated.

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Single colloidal crystals

Noel A. Clark & Alan J. Hurd

Department of Physics and Astrophysics, University of Colorado, Boulder, Colorado 80309

Bruce J. Ackerson

Department of Physics, Oklahoma State University, Stillwater, Oklahoma 74074

Colloidal suspensions of highly charged, monodisperse polymer spheres exhibit long-range (crystalline) translational ordering in appropriate conditions of charge, number density, counterion concentration and temperature[1–5]. These 'colloidal crystals', which can conveniently be made to have lattice parameters comparable to or greater than optical wavelengths, offer unique opportunities for the study of the collective static and dynamic behaviour of strongly interacting spherical particles. For example, such systems may readily be probed by relatively simple but powerful light scattering spectroscopic techniques[6,7]. Furthermore, the ordering itself offers intrinsic advantages, both experimental (for example, fluctuations normally observable only about the k-space origin appear about Bragg spots, with reduced stray light and multiple scattering effects), and theoretical (the simplicity of calculating on the basis of a known rather than only statistically defined structure is well appreciated from experience with atomic solids and liquids). To exploit these ordered structures fully, reliable methods of producing orientated single crystals suitable for light scattering and other optical studies must be developed. For light scattering from bulk samples the primary requirement is that the ratio of interparticle spacing to diameter, a/d, be sufficiently large. This reduces interference by multiple scattering and renders the particle scattering factor nearly constant through those scattering angles [$|k| < (2 \rightarrow 5)2\pi/a$] where the most useful information concerning crystal structure and dynamics appears. We now describe a technique whereby single body-centred cubic (b.c.c.) colloidal crystals, well suited for light scattering and having particular orientations, may be produced.

Our method is shown schematically in Fig. 1, and may be described as follows. The colloidal suspension (0.109 or 0.234 μm diameter Dow polystyrene spheres at ~0.1 wt% in thoroughly deionised water) is introduced into a rectangular quartz cell of path length 1 or 2 mm (Fig. 1*B*). A shear flow is created by mechanically rocking the cell about the axis normal to the flat quartz plates which form the faces of the cell. The intensity distribution of light scattered by the spheres in suspension from a weakly focused argon ion laser is observed and photographed on a translucent screen. At zero flow rate the suspension, if suitably deionised, will crystallise. The application of shear flow disrupts the crystallisation such that suspensions which are crystalline at low or zero shear rates adopt a liquid-like, amorphous structure at sufficiently high shear rates (≳20 Hz). Figure 2*A* shows the scattered light intensity distribution from a suspension under shear and it exhibits the diffuse Debye–Scherrer ring typically observed in the static structure factor of liquids of strongly interacting spherical particles. If the shear flow is stopped abruptly (within 1 s), crystallisation begins immediately, with the nucleation of crystallites at a large number of (as yet unidentified) sites in the suspension which grow to fill the entire suspension volume in a few minutes. The result is a polycrystalline array yielding a scattered light intensity distribution typical of crystalline powders (Fig. 2*B*). Such arrays seem to be stable over periods of several months.

If, on the other hand, the shear rate is only very gradually decreased to zero (over a period of ~10^3 s), the resulting structure is much more highly ordered, as indicated by its diffraction

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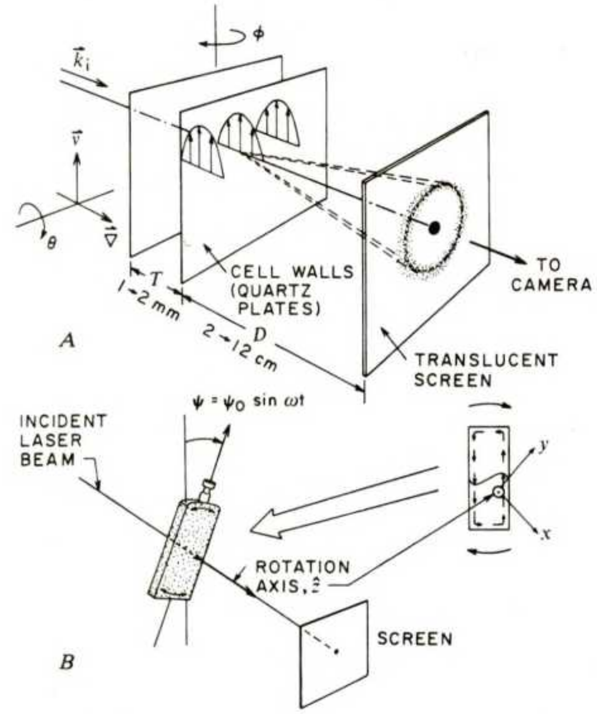

Fig. 1 *A*, Geometry of the apparatus for the generation and observation of b.c.c. single crystals. *B*, Details of the sample cell. Samples were placed in quartz cuvettes of dimension 1 or 2 mm × 5 mm × 10 mm, with a small amount of mixed bed H-OH ion-exchange resin in the bottom. Variable shear flow was induced by rocking the cell about the z axis ($\psi \cong \psi_0 \sin \omega t$) at a variable frequency ω. Inertia of the suspension produced the indicated flow pattern relative to the cell, with $\vec{v} \perp \hat{z}$ in the probed region and with the largest velocity gradient having $\vec{\nabla}| |\hat{z}$. The $\hat{x}$ and $\hat{y}$ axes in the inset are shown in one of three possible orientations, the $\hat{x}$ axis of crystals making angles of 0°, 60° and 120° to $\vec{v}$.

pattern (Figs 2*C*, 3*A*). The study of this pattern and its dependence on the cell orientation angles (θ, φ, Fig. 1*A*) indicates that this structure is an array of two-dimensional hexagonal closest packed (h.c.p.) layers orientated normal to the velocity gradient (z axis, that is, parallel to the quartz plates) and with a set of two-dimensional h.c.p. rows of index 10 parallel to the velocity vector. We believe the stacking of these h.c.p. layers to be nearly random as, on the one hand none of the Bragg spots in the two-dimensional h.c.p. pattern is eliminated by this stacking, but on the other hand the two-dimensional h.c.p. 30 spots are generally of comparable intensity to the others, indicating the lack of registration of the two-dimensional h.c.p. planes in discrete three-dimensional h.c.p. sites. The formation of this and other structures of intermediate order from the liquid as the shear rate is lowered will be discussed elsewhere.

This disordered array of two-dimensional h.c.p. layers is a metastable structure. Within several hours of its formation a new structure appears at sparsely scattered nucleation sites and slowly grows over a period of days to weeks to fill the suspension volume. Figure 3*B* shows a typical diffraction pattern obtained from this second structure with the cell normal to the incident laser beam (θ. $\varphi = 0$). We identify the second structure as a single crystal orientated as indicated in Fig. 4*A*, with a set of planes containing a 110 and a 100 axis parallel to the two-dimensional h.c.p. planes of the metastable structure and therefore parallel to the bounding plates. The b.c.c. structure has been observed previously in low-density colloidal crystals[5]. The nucleation process is such that the b.c.c. 110 axis is parallel to one of the three two-dimensional h.c.p. 10 axes, allowing three distinct orientations of the b.c.c. crystals about the z axis. The b.c.c. crystals, once nucleated, grow most rapidly along the z axis to fill the space between the plates and then gradually expand in the plane of the cell, with large crystals growing at the expense of smaller crystallites. The result, after several days to weeks, is a mosaic of single b.c.c. crystals, each completely filling the millimetre-size gap between the cell walls, and having one of the three preferred orientations about z. In a sample left undisturbed for 2 months after preparation of the h.c.p. structure single b.c.c. crystals as large as 7 mm × 5 mm × 1 mm were found.

Figure 4 *A* shows the b.c.c. crystal orientation which produces the intensity distribution of Fig. 3*B* along with the corresponding f.c.c. reciprocal lattice and Ewald sphere for our light scattering geometry with the sample cell normal to the laser beam (θ, $\varphi = 0$). By suitably rotating the sample about the x and y axes some 16 reciprocal lattice points can be brought onto the Ewald sphere to produce Bragg scattering. Figure 3*C* shows typical results. Here the cell has been rotated about two axes ($\theta = -8.1°$, $\varphi = -11.5°$) to bring the 011, 211 and 200 points onto the Ewald sphere ($2\pi/k_i = (\lambda/n)i = 458$ nm$/1.33 =$ 344 nm). Note the complete absence of 111 and 100 spots, which disappear in b.c.c. diffraction patterns, whereas the observable Bragg reflections index to within a few per cent of those expected for a b.c.c. lattice. Typical cubic lattice parameters were $a = 2.2$ μm, about 10 particle diameters.

The geometry of Fig. 4*B* indicates that there is no Bragg scattering with the cell normal to the incident beam, so that the intensity distribution of Fig. 3*B* must arise from displacements of spheres from their positions in a perfect b.c.c. lattice. Visual inspection of this light shows a pattern of fluctuating coherence areas indicating that it is dynamic in character. Preliminary intensity fluctuation spectroscopy studies show a characteristic fluctuation time which increases as the intensity increases, such as when a Bragg reflection is approached. These observations, along with the similarity of the observed intensity distribution to that for X-ray thermal diffuse scattering in the same planes of atomic b.c.c. crystals[8], lead us to identify this non-Bragg scattering tentatively as thermal diffuse scattering, that is, arising from thermal vibrations of the b.c.c. lattice.

The quality of the b.c.c. single crystals was evaluated by (1) monitoring the presence or absence of spurious Bragg spots; (2) monitoring the spatial distribution of the Bragg reflected light; (3) by the presence of Kossel lines. The b.c.c. crystals, once formed, were extremely delicate. Motion of the cell would invariably lead to distortion and shrinkage of the crystals. Damage produced by motion of the cell, if not too extreme, would anneal out over a period of a few days. In carefully handled samples diffraction-limited Bragg spots from crystals of size ~1 mm³ could be readily obtained.

In well ordered samples the diffraction pattern exhibited Kossel lines (Fig. 3*D*). These are light (dark) bands in the diffusely scattered light where rays have been Bragg reflected into (away from) directions corresponding to the Bragg angle for a set of crystal planes. The diffusely scattered light is incident at

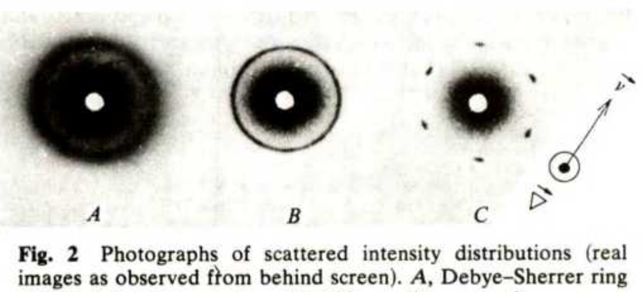

Fig. 2 Photographs of scattered intensity distributions (real images as observed from behind screen). *A*, Debye–Sherrer ring obtained for a suspension of 0.109 μm-diameter spheres translationally disordered by shear at a root mean square shear rate of 20 Hz: (θ, $\varphi = 0$). This suspension is crystalline at zero shear. $T = 1$ mm, $D = 2$ cm. *B*, Crystal powder pattern obtained from the suspension of *A* if the shear is abruptly stopped; (θ, $\varphi = 0$). Intensity distribution obtained from suspension of *A* if the shear rate is gradually lowered to zero. The shear velocity and gradient directions are indicated. The structure is a disordered array of two-dimensional h.c.p. layers with the two-dimensional h.c.p. planes normal to $\vec{\nabla}$ and having a 10 direction normal to $\vec{v}$ (θ, $\varphi = 0$). $T = 1$ mm, $D = 12$ cm.

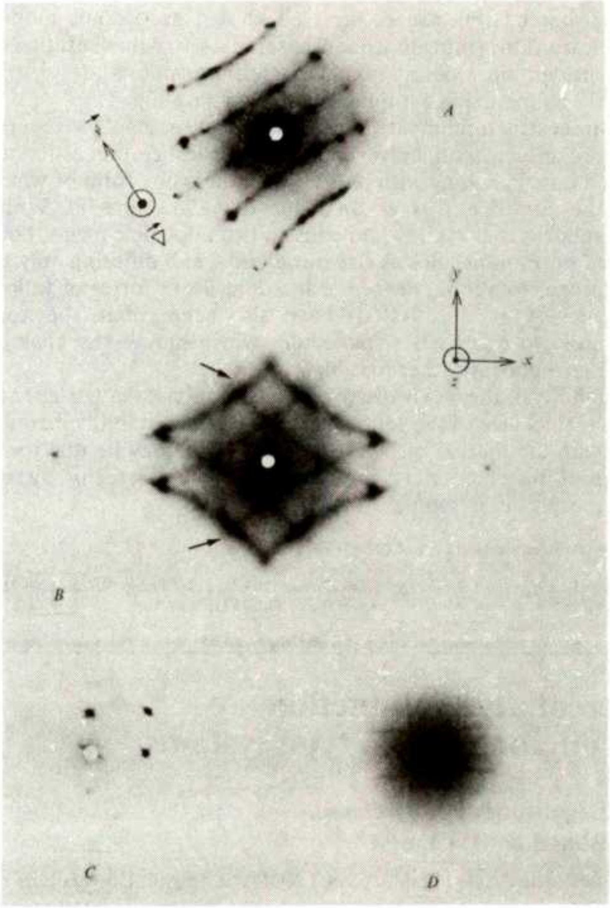

Fig. 3 Photographs of scattered intensity distributions (real images as observed from behind screen). *A* Same as Fig. 2 *C* for 0.234 μm diameter spheres $[(2\pi/k_i)=386\ \mathrm{nm}]$. *B*, Intensity distribution observed for $(\theta, \varphi=0)$ for one of the three single b.c.c. crystals that can nucleate from the structure of Fig. 3*A*. This scattered light is thermal diffuse. The Kossell lines (indicated by arrows) are modifications of the thermal diffuse intensity along pairs of parabolas symmetrically arranged as mirror reflections about the origin, each pair arising from a reciprocal lattice vector and its negative $[(2\pi/k_i)=386\ \mathrm{nm}]$. *C*, Crystal of Fig. 3*B* rotated to $(\theta=-8.1°, \varphi=-11.5°)$ to yield the 011, 211 and 200 Bragg reflections. The b.c.c. 011 spacing is the same as the two-dimensional h.c.p. 10 spacing $[(2\pi/k_i)=344\ \mathrm{nm}]$. *D*, Kossel lines obtained by illumination of a single b.c.c. colloidal crystal orientated as in *B* with a diffuse point source made by focusing the incident laser beam onto a thin Teflon sheet as it enters the cell $(d=0.234\ \mu\mathrm{m}, 2\pi/k_i=386\ \mathrm{nm})$.

all angles to the crystal so the affected rays lie on a cone coaxial with each reciprocal lattice vector (normal to the crystal planes) and at an angle complementary to the Bragg angle[9]. The Kossel lines occur at the parabolic and hyperbolic intersections of these cones with the viewing screen. They appear in X-ray scattering studies of good single crystals and are a sensitive although qualitative indicator of crystal perfection. Motion-induced distortion of a b.c.c. single crystal which has no apparent effect on the Bragg or thermal diffuse scattering can completely eliminate the Kossel lines, which will gradually reappear over several days if the sample is left untouched. The Kossel lines can be viewed alone by illuminating the crystal with diffuse light from a point source, obtained by placing a thin Teflon scatterer in the focused incident laser beam where it enters the cell. The result is shown in Fig. 3*D*.

The b.c.c. crystalline structure bears common features with the randomly stacked two-dimensional h.c.p. from which it nucleates, as indicated in Fig. 4*D*. Both structures are composed of a stack of layers which are parallel to the bounding plates. In the first structure these layers are two-dimensional h.c.ps. In the b.c.c. structure they are distorted two-dimensional h.c.ps, having been stretched in only one dimension, that which becomes the b.c.c. 100 axis (Fig. 4*C*). There seems to be little or no distortion along the other direction (b.c.c. 110 spacing = two-dimensional h.c.p. 10 spacing). The appearance of the b.c.c. is thus accompanied by the disappearance of rows of particles in the two-dimensional h.c.p. layers, perhaps by dislocation motion. If we assume that the overall particle number density remains constant during the transition to b.c.c., the decrease in the number of particles per unit plane area must be accompanied by an increase in the number of planes between the cell walls. For zero density change the two-dimensional h.c.p. layer spacing, c^*, would have to be $(3^{1/2}/2)a$ for a c^*/a^* ratio of 1.83. This somewhat large value may be a result of the shearing which tends to force the spheres into planes normal to the flow gradient. Once the flow is stopped the spheres become too densely packed into layers which are too far apart, and move to form a more energetically favourable structure.

In the conditions of our experiments the crystals were only a few degrees kelvin below their melting temperature, as large room temperature variations would cause partial melting. Recently it has been shown on the basis of an extended Landau theory that, near a weakly first order melting, crystals of spherical particles should exhibit a b.c.c. structure[10]. Our observation of a transition from a metastable two-dimensional h.c.p. layer array structure to a b.c.c. structure provides additional evidence to support this argument.

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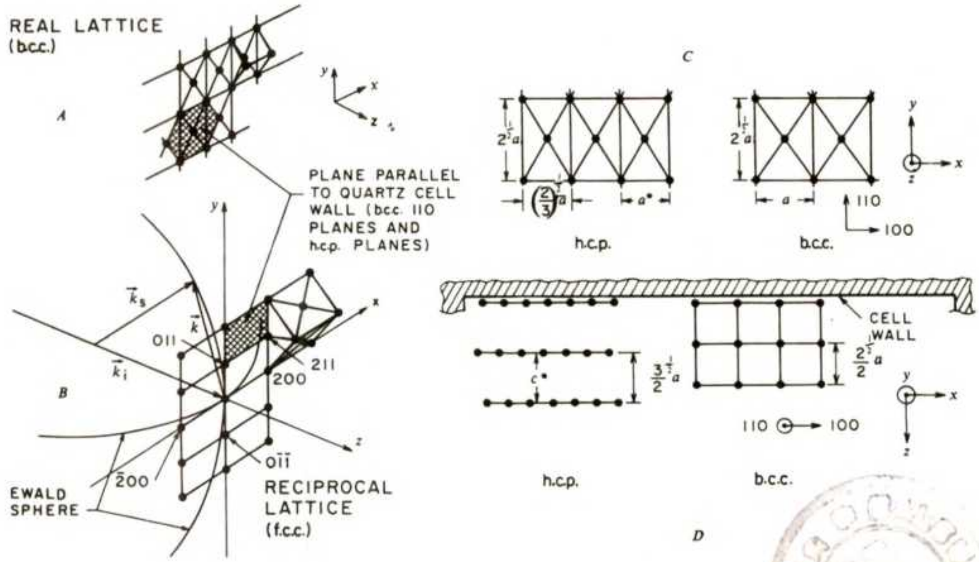

Fig. 4 *A*, b.c.c. crystal orientation which produces patterns of Fig. 3*B*,*C*. *B*, Corresponding f.c.c. reciprocal lattice and Ewald sphere. *C*, Stretching transformation which must occur in the two-dimensional h.c.p. layer during the growth of the single crystal b.c.c. structure. *D*, Randomly indexed h.c.p. layers of spacing $c^*=(3^{1/2}/2)a$ transform into layers at the b.c.c.-indexed positions with spacing $(2^{1/2}/2)a$.

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A new phase in solid state arsenic

A. N. Rodionov, R. I. Kalendarev, G. V. Tchikvaidze & J. A. Eiduss

Peter Stuchka Latvian State University, Riga, USSR

It has generally been considered that in the solid state, arsenic exists as four allotropic modifications—yellow (As(I)), amorphous (As(II)), orthorhombic (As(III)), and rhombohedral (As(IV)). Free energy content is lowest for the rhombohedral form. The other forms are metastable, the yellow form being the least stable, existing only at low temperatures and in the dark[1]. It is the yellow form which has been the least studied. Since the effect of temperature, as well as that of irradiation with light or X rays, is to induce amorphisation in yellow arsenic, no X-ray structural data are yet available, and only indirect observations have been available. From these a cubic form has been tentatively assigned to yellow arsenic crystals. Spectroscopic data suggest molecular bonding in the crystal, with the As_4 molecule as the structural unit—a molecule previously observed in the vapour state[2].

There is little information available on the thermodynamic properties of yellow arsenic. This is relevant to attempts to raise the thermal stability margin, thus opening the possibilities of practical application of the material for silverless photography. We have now designed and built a Calvet-type microcalorimeter for studying phase transitions in thin evaporated films. Using this device for differential thermal analysis (DTA) we have obtained DTA curves showing the existence of a previously unknown reversible (endothermic) phase transition within the metastable As(I) yellow form of arsenic which thus contains two states As^1(I) and As^2(I).

The transition curve for As(I) starts at 227 K, reaching a maximum at 230 K. Reversibility was tested by repeated heating–cooling cycles of the calorimeter, the heating being interrupted immediately after termination of endothermic phase transition.

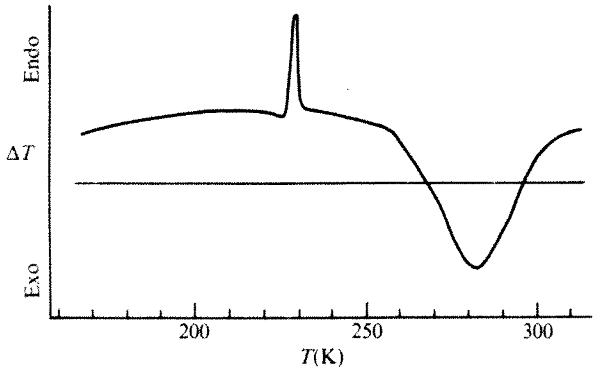

Fig. 1 DTA curve of thin evaporated film of yellow arsenic condensed at 205 K.

Further heating causes the well known exothermic monotropic transition into amorphous arsenic As(II) with liberation of a considerable amount of heat. Both transitions are clearly visible on the thermographic curve shown on Fig. 1.

Direct structural measurements of yellow arsenic have so far proved unsuccessful, however, we can suggest certain estimates based on an analogy with phosphorus, the white form of which can be considered as an analogue of yellow arsenic. White phosphorus is also known to contain two allotropic forms, both based on P_4 molecules as structural units, and differing only by additional rotational degree of freedom. Both forms of yellow arsenic—As^1(I) and As^2(I)—have also been found spectroscopically to contain As_4 molecules which makes the analogy with phosphorus rather feasible.

Stability of the newly discovered yellow phase As^2(I) depends on the thickness of the yellow arsenic layer, the stability increasing with diminution of layer thickness. This may be due to an increase in surface energy with thinning of layer, leading to a rise in amorphisation temperature.

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Percolative conduction in microemulsion type systems

B. Lagourette, J. Peyrelasse, C. Boned & M. Clausse*

Université de PAU et des PAYS de l'ADOUR, Institut Universitaire de Recherche Scientifique, Département de Physique, Laboratoire de Thermodynamique, Avenue Philippon, F–64000 PAU France

In the presence of a suitable combination of surfactants (an alkaline metal soap and a medium chain length alcohol, for example), water and oil type organic liquids can form transparent compounds of low viscosity that have been labelled "microemulsions", with little or no mechanical agitation (spontaneous emulsification) (refs 1–3). Depending on the chemical nature of the surface active agents and the relative constituent proportions, water-in-oil (w/o) or oil-in-water (o/w) systems can be obtained. (Because of the similarity between tertiary solutions of inverted micelles and w/o microemulsions, the term 'inverted micellar solutions' has also been suggested[4–7].) Microemulsions have been investigated by many scientists interested in liquid state and surface physicochemistry and by many technologists foreseeing numerous applications in industry[8,9]. It has been suggested[9–13] that conductivity and permittivity studies could provide, along with other techniques, valuable information about the structure and phase behaviour of microemulsions which are considered to consist of dispersions between a few tens and a few hundreds of angströms in diameter globules made up of an inner spherical core surrounded by a concentric shell of mixed surfactant and cosurfactant[1–10]. The experiments reported here show that the conductive behaviour of certain microemulsion systems can be accounted for using the percolation and effective-medium theories[14–16] that have depicted transport properties and continuous metal–non-metal transitions in disordered materials with microscopic inhomogeneities associated with, for example, density, composition[15] or bonding configuration[17] fluctuations. This result, which could help in understanding the structural behaviour of microemulsions, is considered in connection with the postulated existence in liquid systems of equilibrium bicontinuous structures, a state described by Scriven[18] as 'related to ordinary liquids as porous media are to homogeneous solids'.

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* To whom correspondence should be addressed.

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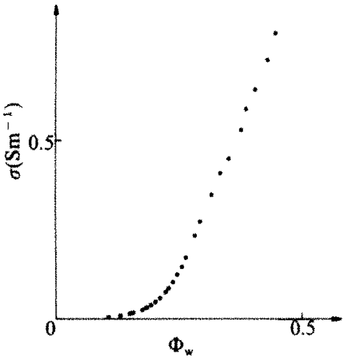

Fig. 1 Variations of σ with Φ_w. The mass fraction of combined surface active agents in the systems was kept at 40%.

Using a Wayne-Kerr B331 autobalance precision bridge, accurate conductivity determinations were obtained at 1.59 kHz and 295 K on w/o type microemulsions involving water and toluene and in which the mass ratio of surfactant (potassium oleate) to cosurfactant (butanol) was kept at 0.5. Unlike previous investigations[9-12,19], measurements were carried out run by run, each run corresponding to a definite class of systems characterised with a fixed value of the mass fraction of combined surface-active agents.

Figure 1 shows the variations of the conductivity σ versus Φ_w, the disperse water volume fraction, recorded on microemulsion systems in which the mass fraction p_s of combined surface-active agents was kept at 40%. Similar plots were obtained on systems characterised by different values of p_s ranging from 30 to 55%. Figure 1 shows that the σ versus Φ_w plot exhibits features characteristic of percolative conduction, the σ experimental values sharply increasing as Φ_w passes a critical value. Kirkpatrick[14] developed, through Monte-Carlo numerical simulations applied to resistor network models, a tractable approach towards percolative conduction phenomena in inhomogeneous media. For conductor–insulator composite materials in which a rigorous percolative conduction is assumed, the effective conductivity σ, zero as long as the conductor volume fraction Φ is smaller than a critical value Φ^P, called the percolation threshold, suddenly takes non-zero values when Φ becomes slightly greater than Φ^P and then increases with Φ. In the vicinity of Φ^P, the dependency of σ on Φ ($\Phi > \Phi^P$) can be demonstrated in the case of three-dimensional systems, by the following scaling law:

$$\sigma(\Phi) \propto (\Phi - \Phi^P)^{8/5} \tag{1}$$

For higher values of Φ, the variations of σ on increasing Φ can be fitted using a relation

$$\sigma(\Phi) \propto (\Phi - \Phi_c) \tag{2}$$

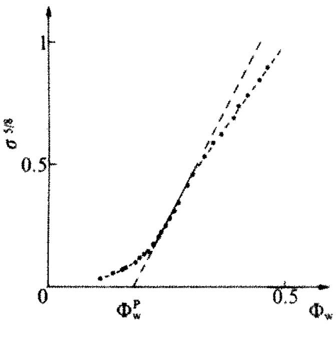

Fig. 2 Determination of Φ^P_w, the water volume fraction corresponding to the percolation threshold, using a scaling law. Specifications as in Fig. 1.

whose range of validity overlaps that of equation (1). It has been shown[14,16] that the B. B. L.[20-22] effective medium formula

$$\frac{\sigma - \sigma_2}{3\sigma} = \frac{\sigma_1 - \sigma_2}{\sigma_1 + 2\sigma}\Phi \tag{3}$$

an expression of the conductivity σ of a binary composite as a function of the conductivities σ_1 and σ_2 of the two of its constituents (Φ being the volume fraction of constituent 1) yields for vanishing values of σ_2 (for conductor–insulator mixtures) an equation of type (2)

$$\sigma/\sigma_1 = \tfrac{3}{2}(\Phi - \tfrac{1}{3}) \tag{4}$$

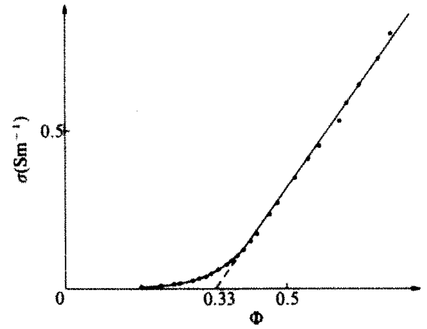

Fig. 3 Variations of σ *with* Φ, the volume fraction of disperse phase (water cores plus shells of combined surface active agents). Specifications as in Fig. 1.

in which Φ_c appears to be 1/3. As mentioned by Kirkpatrick[14], different theoretical values have been suggested for percolation thresholds in three-dimensional systems depending on their geometry. The value $\Phi^P = 0.29$ is of particular relevance to microemulsion studies as it was derived from numerical estimations on a continuum percolation model in which the allowed volumes surrounding percolation sites are assumed to be identical spheres that can overlap and have centres distributed at random. This model is consistent with that inferred from other investigations into microemulsion structure and internal interactions[1-10,19,23]. In the present study, the percolation threshold was determined in terms of water volume fraction by plotting, in accordance with equation (1), $\sigma^{5/8}$ versus Φ_w. Figure 2 shows that the middle part of the plot can be fitted by part of a straight line whose intersection with the Φ_w axis provides Φ^P_w, the critical water volume fraction whose mean value was found to equal 0.176 ± 0.005. Comparing this experimental determination with the theoretical threshold value of 0.29 mentioned above yields the ratio of the disperse globule external radius, a, to the water core radius, a_c, namely $(a/a_c) = 1.18 \pm 0.01$. This value is in excellent agreement with that reported by Lagües *et al.*[19] who found, through neutron scattering measurements, $a/a_c = 1.17$ in the case of water-in-cyclohexane microemulsions using SDS and 1-pentanol as the surface-active agents. Lagües *et al.* suggested for their systems a water critical volume fraction of 0.078. Assuming that the value of the ratio (Φ/Φ_w) as determined from that of (a/a_c) remains unchanged at higher water contents, it was possible to plot the conductivity data versus Φ, the volume fraction of the disperse phase (water cores plus combined surfactant shells), to check whether the microemulsion conductive behaviour beyond the percolation threshold could be fitted by equation (4). Figure 3 shows that, for values of $\Phi > 0.4$, the σ versus Φ diagram is part of a straight line whose intersection with the Φ axis gives $\Phi_c = 0.330 \pm 0.005$, which accords with the value of 1/3 predicted by equation (4). A similar agreement was observed for the other values of p_s. Moreover, whatever the value of p_s, the values $\sigma(1)$ obtained by extrapolating the σ versus Φ plots at $\Phi = 1$ allow values of the slope factor S to be computed which are in excellent agreement with the theoretical value of 3/2 in equation (4); for instance, the value 1.31 S m⁻¹

deduced from Fig. 3 yields $S = 1.51 \pm 0.01$. This general result proves that $\sigma(1)$ can be considered as representative of the conductivity σ_1 of the conducting constituent in the systems, before their inversion into o/w type ones. Note that from Figs 2 and 3, below Φ^P, the conductivity is not strictly equal to zero as it should be were a rigorous percolative conduction exhibited by the microemulsions studied. The non-zero residual conductivity below the percolation threshold could be accounted for on the basis of electrophoretic movements of the disperse globules, as suggested by Peyrelasse *et al.*[24].

These results show that the percolation and effective-medium theories, that are usually applied to semiconductor–metal transitions observed in inhomogeneous systems and to transport properties in amorphous solids, can be extended to the case of microemulsion electrical behaviour. General descriptions of the topological aspects of percolation phenomena in inhomogeneous media have been given by Kirkpatrick[14] and Webman *et al.*[16]. In a conductor–insulator composite, the conducting constituent is divided into isolated clusters as long as its volume fraction Φ remains $<\Phi^P$, bulk conduction phenomena being so inhibited. When Φ reaches and slightly exceeds Φ^P, a certain number of clusters interconnect with each other leading to the formation of a large cluster acting as a conducting path stretched throughout the sample that therefore acquires a non-zero conductivity. When Φ is $>\Phi^P$, the large cluster extends rapidly, by absorbing small isolated clusters which then become less and less, and the sample conductivity increases proportionally to Φ. This scheme may be tentatively applied to the case of microemulsion electrical conductivity, on the basis of the present experimental results. The increase in microemulsion conductivity observed when the water content is raised above the percolation threshold may result from a progressive aqueous droplet interlinking and clustering process. The fact that microemulsion conductive behaviour at higher water contents can be described by the effective-medium theory equation (4), in which the values of Φ are related to those of Φ_w with the constant ratio 1.18 as determined at the percolation threshold, suggests that the clustering process does not affect the membrane-to-water phase volume proportion. This result appears to be an extension of that reported by Lagües and coworkers[19,23] as to the constancy of (a/a_c) with Φ_w increasing up to 0.3 in water-in-cyclohexane microemulsions. Note that the present experiments yield an interesting idea as to the conduction mechanism in microemulsions. The fact that, p_s being fixed, σ increases with Φ while σ_1 remains constant proves that the conduction mechanism taking place in percolating microemulsions is essentially interfacial, as also suggested by Lagües *et al.*[19]. In support of this hypothesis, note that σ_1, which was found to increase proportionally to the amount of surfactant, exhibits values that are lower than those of the corresponding KC1 aqueous solutions. For instance, the value of σ_1, as deduced from Fig. 3, is $1.31\ \mathrm{S\ m^{-1}}$ while the aqueous solution with the same molar fraction of solubilised KC1 would display a conductivity of $4\ \mathrm{S\ m^{-1}}$ or so.

The droplet interlinking and clustering process suggested by the percolative behaviour of microemulsion conductivity might be predictive of the phase inversion phenomenon, the w/o to o/w transition taking place through the constitution of intermediary equilibrium states of the bicontinuous type[18], a statistical model of which was set up by Talmon and Prager[25] who treated 'the microemulsion structure as a random geometry of interspersed oil and water domains generated by a Voronoi tesselation', the surface active agents being assumed to be entirely adsorbed at the water/oil interface.

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Primate facial allometry and interpretations of australopithecine variation

Robert S. Corruccini

Department of Anthropology, Southern Illinois University,
Carbondale, Illinois 62901

Russell L. Ciochon

Department of Sociology and Anthropology, University of North
Carolina, Charlotte, North Carolina 28223

Pilbeam and Gould[1] have discussed African Plio-Pleistocene hominid evolution in the context of allometry (size-dependent morphological change). These authors demonstrate that some general aspects of australopithecine morphology (tooth, brain and body size) support the hypothesis that certain early African hominids were merely scaled variations of each other at different sizes. They also speculate that the methods applied to these very broad anatomical categories can be extended to more specific and detailed traits, especially in the face and cranium. Such traits underlie most taxonomic and phylogenetic discussions of the early African Hominidae, so it is useful to follow Pilbeam and Gould's lead, as we do here, and investigate the structural differences in the australopithecine face and cranium in a quantifiable fashion.

We chose 16 standard measurements of the face and its relation to the neurocranium provided by Tobias[2] for the best preserved australopithecine specimens: *Australopithecus boisei*, represented by Olduvai Hominid 5 (OH 5), and *Australopithecus africanus*, represented by Sterkfontein 5 (Sts 5). The measurements have also been taken on other available australopithecine material (SK 48 and especially KNM-ER 406) although these are less complete and not as well described as the two crania that will be the focus of our analysis. The measurements are listed in Table 1.

Earlier allometric studies of primates[3–12] have relied on bivariate comparisons of measurements. As it is difficult to synthesise the resulting patterns when multiple measurement combinations are involved, we use a multivariate method that allows the growth pattern to be represented as a coherent, covarying entity. A principal component model[13] is available for this purpose but is better suited to intraspecific studies than to the interspecific approach which, like Pilbeam and Gould, we shall use. Thus, for the two fossils, a vector of allometric

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coefficients (k) can be computed that connects the two points in the logarithmic hyperspace defined by the 16 dimensions[14]:

$$k_i = \frac{(\log x_{i1} - \log x_{i2})p^{1/2}}{[\sum_{j=1}^{p} (\log x_{j1} - \log x_{j2})^2]^{1/2}}$$

where p is the number of measurements and x the array of measurements. This two-dimensional solution is automatically of rank one. These coefficients, which describe the relative changes in proportions with increasing size in the two australopithecine morphs, are compared with the characteristic allometry between primate species. A somewhat novel sampling design is used for non-hominid primates. If all primates were to be treated as a single sample, inherent taxonomic differences which happen to coincide with size changes would be mistakenly identified as allometric in nature. For example, prosimians have quite elongated faces and snouts as well as generally small body size when compared with anthropoids, but this difference is not allometric. Therefore, we have constructed a sample of pairs of closely related but size-differentiated primate taxa, just as we are comparing australopithecine species. The statistical properties of pairwise allometric vectors can be compared with australopithecine values.

For each sampled species the modal-sized individual of five randomly selected male and five female specimens (or less depending on availability) was measured. Each complete pair of taxa yields four vectors, compared between the male and female of one to the two typical individuals of the other. The 12 species pairs result in a sample of 33 allometry vectors that in turn was visually selected from 200 specimens. The dimorphic species pairs are: *Indri indri, Avahi laniger*; *Cheirogaleus major, Microcebus murinus*; *Lemur variegatus, Lepilemur mustelinus*; *Galago crassicaudatus, Galago demidovii*; *Perodicticus potto, Loris tardigradus*; *Saguinus midas, Cebuella pygmaea*; *Ateles belzebuth, Saimiri sciureus*; *Nasalis larvatus, Presbytis melalophus*; *Erythrocebus patas, Cercopithecus talapoin*; *Papio cynocephalus, Macaca fascicularis*; *Hylobates syndactylus, Hylobates klossii*; *Pan gorilla, Pan troglodytes*. The sample pairs consists of four species of genera, six genera within subfamilies and two representatives of the large and small endpoints of families. The exact level of taxonomic distinctness between robust and gracile australopiths remains controversial[15–17], but is probably less than some of the intergroup differences in this comparative sample. Nevertheless, the variation that is non-allometric among the species pairs will be random and non-cumulative compared with that resulting from the consistent size discrepancy.

The allometries are shown in Table 1. Among primates in general, facial breadths are negatively allometric to superior facial length (that is large primates have disproportionately long faces); alveolar height is highly variable; nasal aperture width is positively allometric to its height, but nasal bone width is negative to length; neurocranial measurements are generally negatively allometric to facial measurements, and cranial lengths are positive to the breadths; the foramen magnum area, which is related to brain size[18], has a relatively invariant relationship to other measurements and shows a value near the 0.66 scaling coefficient of brain to body which is characteristic of primates as a whole[12].

Between *A. africanus* (Sts 5) and *A. boisei* (OH 5), shape differences in facial height and the first four parameters of facial breadth co-vary closely with the interspecific primate allometries. This signifies that general primate growth tendencies do satisfactorily explain shape differences within the coronal plane of the face of australopithecines. The relatively narrow nose of the OH 5 specimen, on the other hand, is contrary to the primate trend, and the coefficient for nasal width falls outside the 99% confidence limits for the primate sample. The inferior tapering of the OH 5 nasal bones (characteristic also of other *boisei* and *robustus* specimens) is a unique phenomenon which is in no way accounted for by allometry. The only living primate in which a similar condition exists is the Asian colobine, *Rhinopithecus*. The longer nasal bones of OH 5, however, are again as would be expected from the primate comparative sample. The australopithecine bimastoid (inferior) cranial breadth grows disproportionately relative to other cranial length and breadth measurements but still falls within two standard deviations of the primate mean. The two cranial breadths do not differ nearly as much between australopithecine and primate values as do the three lengths. The small allometry coefficient for horizontal projective length relates to the vertical face and lack of prognathism in OH 5, whereas larger primates are generally more prognathic. This measurement and the related superior length are significantly different in the two lists of coefficients. The average coefficient for facial measurements divided by the average of neurocranial dimensions is 1.75 between OH 5 and Sts 5, indicating the strong rate of allometric increase of face to calvaria. This figure is also positively allometric among primates at 1.26, but the latter value is significantly lower. The smaller foramen magnum of the *A. boisei* specimen also departs sharply from the expected primate value, although cranial capacity itself seems to scale according to the primate norm in all the australopithecines[1].

Table 1 Allometry coefficients comparing facial measurements of two species of *Australopithecus* with the means and s.d. values of primate interspecific allometry coefficients based on the same measurements

Measurement (endpoints)	OH 5-Sts 5	Primates Means	Primates (s.d.)
Inner biorbital breadth (fmo–fmo)	0.64	0.63	(0.19)
Bizygomatic breadth (zy–zy)	0.84	0.80	(0.15)
Maxillary breadth (zm–zm)	0.90	0.89	(0.20)
Anterior interorbital breadth (mf–mf)	1.08	1.07	(0.64)
Superior facial height (n–pr)	1.19	1.10	(0.26)
Cranial length (g–op)	0.49	0.72	(0.17)
Horizontal projective length (pr–i)	0.37	0.87	(0.14)
Superior facial length (ba–pr)	0.35	0.98	(0.14)
Bimastoid breadth (ms–ms)	0.80	0.61	(0.16)
Maximum biparietal breadth	0.39	0.53	(0.16)
Alveolar height (ns–pr)	0.80	0.39	(1.04)
Maximum nasal width	0.28	1.03	(0.20)
Nasal aperture height (ns–rh)	1.03	0.86	(0.33)
Nasal bone inferior breadth	−2.79	0.77	(0.27)
Nasal bone length (n–rh)	1.15	1.28	(0.56)
Foramen magnum (area$^{1/2}$)	−0.18	0.65	(0.11)

fmo, frontomalare orbitale; zy, zygion; zm, zygomaxillare; mf, maxillofrontale; n, nasion; pr, prosthion; g, glabella; op, opisthocranion; i, inion; ba, basion; ms, mastoidale; ns, nasospinale; rh, rhinion.

Thus, there are considerable parallels between the OH 5/Sts 5 australopithecine shape differences and those generally characterising larger and smaller related primates, especially within the face and between the face and neurocranium. However, as previously noted, there are several departures from the primate vector in nasal aperture shape, the nature of the nasal bones, prognathism and foramen magnum area. When the relatively recently discovered East Rudolf 406 robust cranium is substituted for OH 5 in the calculations, identical results to these are obtained except for the larger foramen magnum of ER 406. Swartkrans 48 also yields highly similar allometry coefficients when compared with Sts 5, with the possible exception of aveolar height.

The more general measurements therefore support the hypothesis of Pilbeam and Gould[1] of australopithecine variation, whereas the more specific do not. With regard to the face and cranium, OH 5, KNM-ER 406 and SK 48 in many ways represent scaled-up versions of Sts 5. As the best estimates now indicate that Sts 5 is 0.5–1.0 Myr older than any of these other fossil crania[19–21], our results supplement evidence[22] for evolution of the robust australopithecines in South Africa from antecedent populations resembling *A. africanus* and the subsequent migration of the *A. boisei* stock to East Africa. Further investigation of Johanson and White's recently proposed model[22,23] of early hominid evolution in Africa, using Pilbeam and Gould's allometry hypothesis[1], should include documentation of divergent modes of change between crania of *A. afarensis* with its proposed descendent species *H. habilis* (encephalisation trend) and *A. africanus* (allometric trend).

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Protein deprivation causes reversible impairment of mucosal immune response to cholera toxoid/toxin in rat gut

Wayne S. Barry & Nathaniel F. Pierce

Departments of Medicine, Baltimore City Hospitals and Johns Hopkins University School of Medicine, Baltimore, Maryland 21224

Secretory antibodies may be the major defence against mucosal infections, especially those due to viruses and non-invasive pathogens such as *Vibrio cholerae* and toxinogenic *Escherichia coli*. The high incidence of mucosal infections in malnourished protein-deficient children[1] may result from defective antibody production[2], but evidence for this is conflicting[3]. We report here that protein deficiency markedly impairs the mucosal immune response to cholera toxoid/toxin (CT), a protein antigen, in rats and that this impairment is rapidly reversed by refeeding.

Protein deficiency was induced in 7-week-old Wistar–Lewis or Fischer rats weighing 140 g by feeding a low protein (3.2% casein) diet which was otherwise nutritionally complete (LP). The control diet was similar but contained 24% protein (HP). As rats given LP ate more for their weight than those given HP, the diet of LP rats was limiting only for protein. After 6 weeks of HP diet rats weighed 240 g whereas those given LP diets weighed 125 g (serum protein 69% of HP rats). LP rats had lymphoid atrophy but the intestinal mucosa was morphologically normal and they appeared well. Refeeding with HP after 6 weeks of LP diet produced rapid growth; within 2 weeks serum proteins were normal and 50% of the weight deficit was recovered.

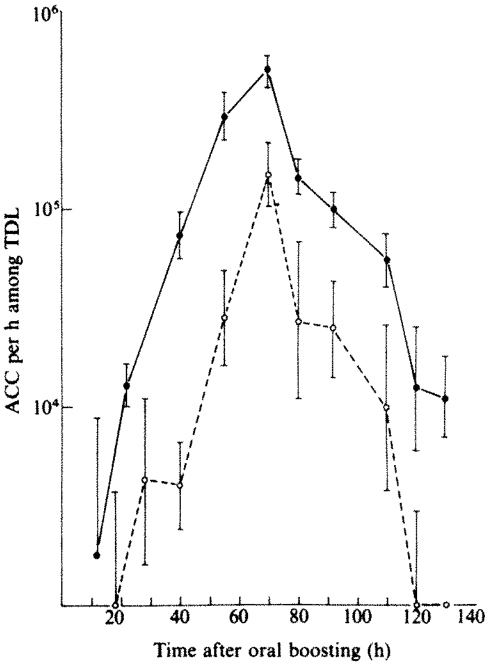

Fig. 1 Antitoxin-containing cells (ACC) among TDL from normal (●) and protein-deficient (○) rats after oral immunisation with CT. Wistar–Lewis rats were begun on test diets at age 7 weeks. For immunisation, CT was given with sodium bicarbonate by intragastric tube to conscious fasting rats. The priming dose was 40 mg crude toxoid (Wellcome) plus 1.5 mg crude toxin (NIH lot 001) given at age 11 weeks. The booster dose was 40 mg crude toxoid given 2 weeks later. These antigens are described elsewhere, as is evidence that this sequence provokes a vigorous mucosal antitoxin response in normal rats[6]. The thoracic duct was cannulated and antitoxin-containing cells among TDL were counted using a fluorescent antibody technique specific for cholera antitoxin[4]. Values are means ± s.e.m. for 3–9 rats given HP or LP diets.

Large thoracic duct lymphocytes (TDL), especially those containing IgA, arise from Peyer's patches and are direct precursors of IgA plasma cells in intestinal lamina propria[4,5]. Table 1 shows the effect of protein deficiency on the number of TDL in unimmunised rats. Diminished numbers of large TDL, including those containing IgA, suggested an impaired mucosal immune response to enteric antigens. This was confirmed in rats immunised orally with CT[6]. HP rats showed numerous antitoxin-containing lymphoblasts among TDL after the second dose of CT; this response was reduced by 73% in rats fed LP 4 weeks before the first dose of CT (Fig. 1). In HP and LP rats more than 96% of antitoxin-containing cells among TDL contained IgA.

Table 1 Thoracic duct lymphocytes (TDL) in unimmunised normal and protein-deficient rats

	Total TDL	Large TDL	TDL containing IgA
HP diet	21	2.1	0.24
	(1.1)	(1.2)	(1.3)
LP diet	9.0	0.61	0.13
	(1.1)	(1.1)	(1.2)
P	<0.001	<0.001	0.06

Fischer rats were given test diets for 6 weeks starting at age 7 weeks. The thoracic duct was then cannulated for collection of TDL. Total TDL and large TDL were determined with a Coulter counter. The number of IgA-containing TDL was determined by autoradiography with ^{125}I-labelled rabbit F(ab')$_2$ anti-rat IgA[4]. Geometric mean cells $h^{-1} \times 10^{-6}$ ($\overset{\times}{\div}$s.e.m.), $n = 10$; *P* values by Student's *t* test.

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The antitoxic response in ileal lamina propria was also impaired in LP rats. Feeding LP diet for only 4 weeks (2 weeks before the priming dose of CT) reduced the number of antitoxin-containing cells in ileal mucosa by more than 90%; however, an LP diet begun with priming was without effect (Fig. 2*a*). The mucosal response was also impaired by about 75% when LP rats were primed parenterally (Fig. 3), indicating that impairment was not due to poor absorption of the priming dose of oral CT. Likewise, the impaired response was unlikely to be due to poor absorption of the booster dose as it was at least 20 times the amount needed to produce a maximum response in previously primed normal rats (N.F.P., unpublished observations).

The possibility that refeeding with HP would correct the impaired mucosal immune response was also studied. Despite having had 6 weeks of LP diet, rats refed 2 weeks before starting enteric immunisation showed normal mucosal antitoxin responses; even when refeeding was begun with enteric priming, substantial recovery of responsiveness occurred (Fig. 2*b*).

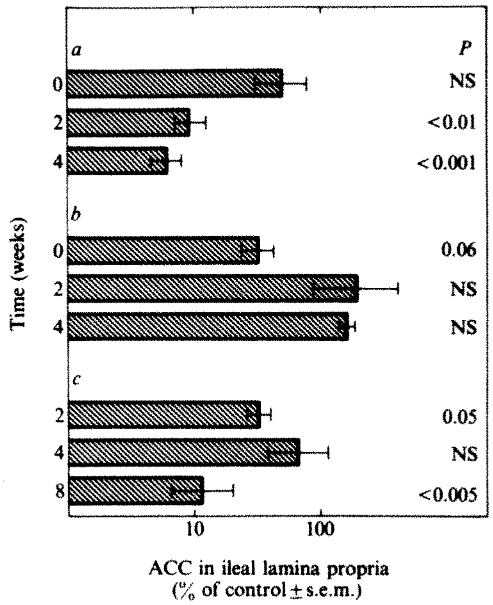

Fig. 2 Antitoxin-containing cells (ACC) in ileal lamina propria of protein-deprived and refed rats. Wistar–Lewis rats were started on LP diets at age 7 weeks. The oral priming and boosting doses of CT are described in Fig. 1 legend. *a*, Time indicates period of LP diet before priming and the LP diet was continued until rats were killed; oral boosting was 2 weeks after priming. *b*, Time indicates period of refeeding before priming. The LP diet was given for 6 weeks, after which HP diet was given. The time of oral priming after refeeding is indicated; oral boosting was 2 weeks after priming. *c*, Time indicates period of LP diet before priming. Oral priming occurred at the indicated interval after starting the LP diet; the diet was continued for 2 weeks after priming; refeeding with HP was then begun and oral boosting was 2 weeks later (4 weeks after oral priming). Scoring of ileal ACC was always 5 days after boosting. Controls were identically immunised and concurrently studied rats given HP diet only. Control responses averaged about 9,000 ACC mm^{-3}. Each group contained 7–17 rats; *P* values were by the *t* test comparing LP rats with concurrently studied controls.

The selective effect of the LP diet on the primary component of the mucosal IgA response was studied by refeeding after enteric priming. Feeding LP for 4 or 6 weeks (2 or 4 weeks before priming), but refeeding with HP 2 weeks before boosting gave a near-normal mucosal antitoxin response; however, extension of the period of LP diet to 8 weeks before priming produced substantial impairment despite refeeding before boosting (Fig. 2*c*).

Thus the ability to mount a specific mucosal IgA response was impaired and corrected by relatively brief periods of protein restriction and refeeding, respectively. The mechanism by which protein deficiency impaired the IgA antitoxin response is unknown and is probably complex. Fewer antitoxin-containing cells among TDL in LP rats indicates that protein deficiency impaired the generation of specific IgA immunoblasts in Peyer's patches[5]; this effect was not specific for antitoxin as non-immune rats had fewer IgA-containing TDL. The greater impairment of the antitoxin response in ileal mucosa (Fig. 2*a*) than among TDL (Fig. 1) suggests that antigen-driven division of immunoblasts in the ileal mucosa[5] was also impaired. Protein deficiency appeared to impair both primary and secondary components of the mucosal immune response; there was direct evidence for an effect on priming (Fig. 2*c*); an effect on boosting is inferred from the shorter period of LP diet required to impair responses in rats not refed before boosting (Fig. 2*a*). The rapid recovery of responsiveness with refeeding parallels the previously described rapid regeneration of atrophic lymphoid tissue when protein-deficient mice are refed[7].

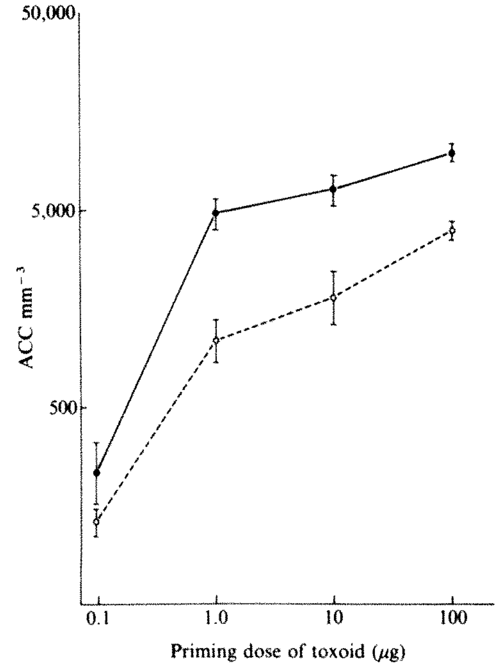

Fig. 3 Effect of protein deficiency on the enteric antitoxin response of rats primed intraperitoneally (i.p.) with purified toxoid. Seven-week-old Wistar–Lewis rats were fed HP (●) or LP (○) diets. Primary immunisation was with purified toxoid (Wellcome) given i.p. (0.1 ml toxoid plus 0.1 ml Freund's complete adjuvant) at age 11 weeks. Intragastric boosting with 40 mg crude toxoid was 2 weeks later. ACC in ileal lamina propria were scored 5 days after boosting; results are geometric mean ± s.e.m., *n* = 5–8 for each value.

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Extra-pituitary inhibition of testicular function by luteinising hormone releasing hormone

A. J. W. Hsueh & G. F. Erickson

Department of Reproductive Medicine, University of California, San Diego, La Jolla, California 92093

Luteinising hormone releasing hormone (LHRH), produced by the hypothalamus, stimulates gonadotropin production by the pituitary[1–3]. Paradoxically, chronic treatment with large doses of LHRH or its agonists causes a decline in testicular steroidogenesis and testicular LH/human chorionic gonadotropin (hCG) receptors; they also cause a decrease in the weight of testes, seminal vesicles and ventral prostate[4–7]. There are three possible mechanisms for the inhibitory action of LHRH and its agonists. At the pituitary level, prolonged action of LHRH or its agonists may make the pituitary refractory to further stimulation by LHRH, with a decrease in serum gonadotropins and subsequent atrophy of the male reproductive organs[8,9]. It is also possible that pharmacological doses of LHRH stimulate the release of a large amount of LH, which in turn 'desensitises' the testis to further LH[10–14]. We have tested a third possibility, namely that LHRH and its agonists act directly on the testis. We report here that LHRH and LHRH agonists cause a decrease in testicular LH/hCG receptors and inhibit testicular steroidogenesis in hypophysectomised male rats, indicating a direct effect on the Leydig cells. This is consistent with our demonstration that LHRH and its agonists act independently of the pituitary to inhibit ovarian steroidogenesis in cultured rat granulosa cells and in hypophysectomised female rats *in vivo*[15].

Immature hypophysectomised male rats were injected daily for 5 d with ovine follicle stimulating hormone (FSH) and/or LHRH analogues or the appropriate vehicle. After hypophysectomy, FSH reduces the general testicular regression and enhances the LH/hCG stimulation of testosterone production[16–19]. Following hormone treatment, animals were killed and testes were removed, decapsulated, weighed and their LH/hCG receptor content and steroidogenic potential determined.

LHRH analogues decreased the testicular weight in both FSH-treated and untreated hypophysectomised rats (Fig. 1*a*). The testes of FSH-treated animals weighed significantly more than the testes of untreated hypophysectomised rats: 139.0 ± 5.1 mg compared with 72.6 ± 1.5 mg (mean ± s.e.m.). Daily injections of [D-Leu^6($N^\alpha Me$)Leu^7]LHRH (analogue I, 200 μg per d) or des-Gly^{10}-[D-Leu^6($N^\alpha Me$)Leu^7,Pro^9-NHEt]LHRH (analogue II, 50 μg per d) decreased the testis weight of FSH-treated animals by 12 and 21%, respectively ($P<0.01$). In untreated hypophysectomised rats, analogue II caused a 20% decrease in testis weight (72.6 ± 1.5 mg to 57.4 ± 2.5 mg; $P<0.01$).

Treatment with LHRH analogues reduced the binding of ^{125}I-hCG to Leydig cells in both FSH-treated and untreated hypophysectomised rats (Fig. 1*b*); binding capacity was 2.1 times greater in the former than the latter. In untreated hypophysectomised rats, analogue II caused a significant decrease in hCG binding ($P<0.01$). Likewise, LHRH analogues I or II given together with FSH significantly decreased FSH-enhanced hCG binding in testicular Leydig cells by 66% and 84%, respectively. Indeed, the effect of analogue II was so great that the testicular LH/hCG receptor content in animals treated with analogue II plus FSH was less than that in animals not treated with FSH ($P<0.01$). Furthermore, LHRH at 50 μg per d also inhibited FSH-enhanced hCG binding by 40%, from 4.21 ± 0.45 ng per testis to 2.51 ± 0.05 ng per testis. The inhibitory effect of LHRH and its analogues was also apparent when hCG binding was expressed as pg hCG bound per mg testis: control, 27.5 ± 1.4; analogue II, 9.6 ± 0.6; FSH-treated, 30.3 ± 3.2; FSH + analogue I, 11.7 ± 1.5; FSH + analogue II, 6.3 ± 0.6; FSH + LHRH, 16.5 ± 0.3.

LHRH analogues also inhibited stimulation of testicular steroidogenesis by hCG. Decapsulated testes were incubated for 3 h at 34 °C in polyethylene vials, half of which contained a saturating concentration of hCG (500 ng ml^{-1}). The androgen concentration in the medium was then determined by testosterone radioimmunoassay[21]. The basal steroidogenic response was low in the control group, and was increased by FSH treatment for 5 d (Fig. 2). In contrast, concomitant administration of analogues I or II together with FSH significantly decreased basal steroidogenesis ($P<0.01$). In the presence of a saturating concentration of hCG, testicular steroidogenesis was stimulated in all groups (Fig. 2, cross-hatched bars). Pretreatment with FSH considerably enhanced the steroidogenic responsiveness of

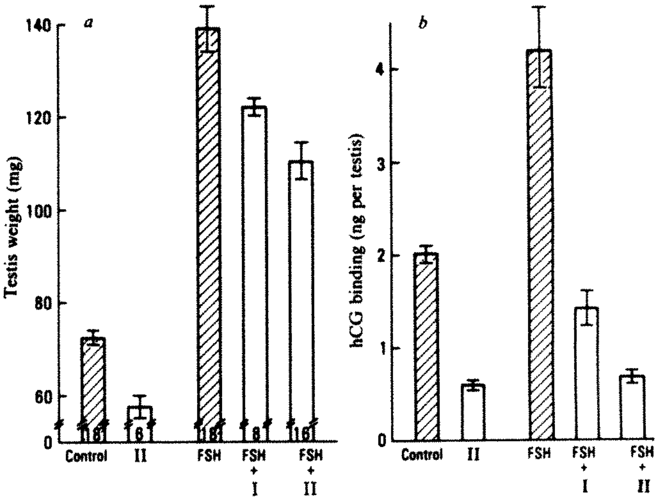

Fig. 1 Effect of treatment with FSH and/or LHRH analogue on testicular weight and ^{125}I-hCG binding capacity of immature (21–23-d-old) hypophysectomised male rats (Hormone Assay Labs). The completeness of hypophysectomy was determined by two parameters. First, 9 d after hypophysectomy of 21-d-old male rats, considerable testicular involution was observed with reduction of testis weight from 169 ± 13 mg to 72.6 ± 1.5 mg. Intact 30-d-old rats have a mean testis weight of 469.4 ± 12 mg. Second, serum LH was measured in hypophysectomised male rats treated with 200 μg LHRH analogue I ([D-Leu^6($N^\alpha Me$)Leu^7]LHRH) or 50 μg analogue II (des-Gly^{10}-[D-Leu^6($N^\alpha Me$)Leu^7,Pro^9-NHEt]LHRH) for 0, 1, 3 and 6 h. In all samples tested, LH in serum was below the limit of detection by LH radioimmunoassay (less than 5 ng NIH-LH-RP-1 per ml). *a*, Beginning 4 days after hypophysectomy, rats received subcutaneous injections daily for 5 d. They received an injection of 50 μg ovine FSH (NIH-FSH-S12) dissolved in 0.1 ml medium 199 with or without an injection of LHRH analogue (200 μg analogue I or 50 μg analogue II) dissolved in 0.1 ml corn oil. Control animals received vehicle alone. Between 20 and 24 h after the last injection, animals were decapitated and their testes were removed, decapsulated and weighed (mean ± s.e.m.). The numbers inside the column indicate the number of testes used. *b*, Decapsulated testes were homogenised in a Kontes ground-glass tissue grinder in ice-cold phosphate buffer (0.01 M, *p*H 7.4) containing 0.15 M NaCl and 0.1% bovine serum albumin (BSA). After centrifugation at 20,000*g* for 15 min, the crude membrane pellet was resuspended to a final concentration of 1 testis per 1.5–3 ml buffer. The concentration of LH/hCG receptors was determined by incubation of serial dilutions of resuspended pellet with a saturating concentration of labelled hCG. Excess unlabelled hCG (100 IU Pregnyl) was added to half of the incubation tubes to obtain nonspecific binding to testis membranes. ^{125}I-hCG (CR-119) was prepared by radioiodination with chloramine T as described previously[20]. The specific activities of the labelled hCG were determined by self-displacement analysis in the radioligand–receptor assay[28] and varied from 20,000 to 80,000 c.p.m. per ng. The fraction of the ^{125}I-hCG able to react specifically with an excess of receptor (that is, the maximum binding activity) was determined for each tracer preparation. This value (45–65%) was used as a correction factor during quantitative analysis of LH/hCG receptor[28]. After incubation of the testicular membrane fraction with ^{125}I-hCG at room temperature (22–24 °C) for 16–18 h, the mixtures were diluted with 3 ml ice-cold buffer and centrifuged at 1,500*g* for 15 min. Pellets were washed once with buffer and then counted in a γ-spectrometer. After correcting for the radioactivity bound to equal aliquots of samples incubated in the presence of an excess of unlabelled hormone, specifically bound radioactive hormone was determined as ng hCG bound per testis. Data represent the mean ± s.e.m. of three experiments.

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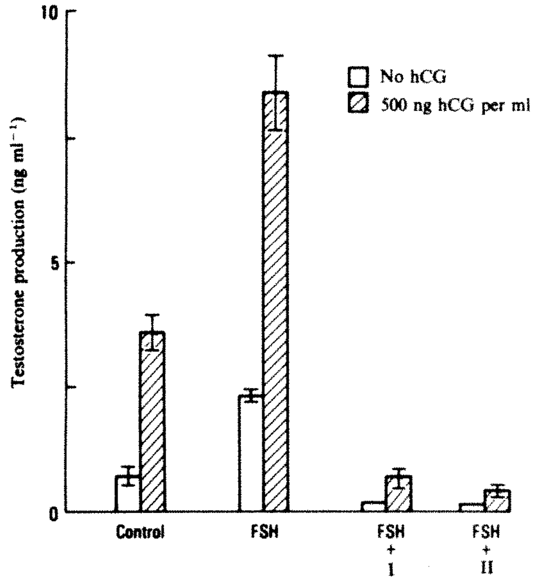

Fig. 2 Effect of treatment with FSH and/or LHRH analogue (I or II) on the steroidogenic capacity of rat testes *in vitro*. Hypophysectomised immature male rats were treated with FSH and/or LHRH analogue as described in Fig. 1. Testes were decapsulated, washed once in ice-cold saline and transferred to polyethylene vials containing 2.0 ml ice-cold medium 199 with 0.1% BSA and 0.1 mM 1-methyl-3-isobutyl xanthine (Aldrich Chemical). Each vial contained one testis, either with or without saturating concentrations of hCG (1,000 ng). All vials were then incubated for 3 h at 34 °C under 95% air, 5% CO_2. Androgen concentration in the incubation medium was measured by specific radioimmunoassay using testosterone antiserum[21]. The antiserum shows no cross-reaction with C_{21} or C_{18} steroids but cross-reacts 100% with dihydrotestosterone, 80% with androstenedione, 30% with 5α-androstanediol and 4% with androsterone. The latter two steroids contribute significantly to total androgen production in immature rats. Three to five samples were used for each point and duplicate determinations were carried out by radioimmunoassay. The results are expressed as mean ± s.e.m.

testes to hCG, whereas a smaller increase was found in untreated hypophysectomised rats. In contrast, concomitant treatment with LHRH analogues I or II markedly depressed the ability of FSH to increase testicular steroidogenic responsiveness to hCG. hCG-induced androgen production in animals treated with FSH plus LHRH analogue is lower than that of control animals receiving no FSH.

These observations indicate that LHRH agonists, in addition to their releasing action at the pituitary gonadotrophs, directly inhibit testicular function. These results may help explain the potent anti-reproductive effect of high doses of LHRH agonists in intact male animals[4-7]. LHRH agonists induce a substantial decrease in LH/hCG receptors as well as decreasing the steroidogenic responsiveness of Leydig cells (Figs 1 and 2). This may, in turn, result in degenerative changes in seminiferous tubules (spermatogenesis), ventral prostate and seminal vesicles[4-7,22]. Although the testis is the most probable site of action of the LHRH agonists, the use of the hypophysectomised male rats in our study does not exclude the possibility that LHRH agonists act on other organs such as the adrenal, resulting in secondary inhibition of the Leydig cells.

The mechanism by which LHRH agonists inhibit Leydig cell function is unknown. Although LHRH binding sites have been identified in the anterior pituitary[23-26], the tissue specificity of the binding has not been demonstrated conclusively. Indeed, Heber *et al.*[27] reported the presence of specific high capacity, low affinity LHRH binding sites in the testis. It is possible that LHRH agonists exert their inhibitory effect through gonadal LHRH receptors. In conclusion, our study provides the first evidence demonstrating an extra-pituitary inhibitory action of LHRH and LHRH agonists on testicular functions in hypophysectomised animals.

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Identified neurones isolated from leech CNS make selective connections in culture

Donald F. Ready & John Nicholls

Department of Neurobiology, Stanford University School of Medicine, Stanford, California 94305

Neurones cultured *in vitro* offer distinct advantages for studying how processes grow towards their targets and form synaptic connections. In contrast to the complex events occurring during the development of the nervous system, synapse formation in culture can be analysed in a few neurones at a time and under controlled conditions[1]. We have now dissected out and cultured single identified neurones from the central nervous system (CNS) of the adult leech. Various types of sensory cells, motor cells, and interneurones can be identified in leech ganglia—each with a stereotyped set of properties, including: (1) the electrical characteristics of its membrane, (2) the arborisation of its branches and the morphology of its terminals and (3) the pattern of connections it makes with other identified neurones, skin or muscle[2]. Thus, cultured cells can be compared in detail with their counterparts *in situ*. We have found that isolated cells survive for several weeks, maintain their membrane properties, sprout and form selective connections.

Leech ganglia were dissected under sterile conditions and placed in culture medium consisting of Leibowitz L-15 and 2% fetal calf serum together with glucose 6 mg ml^{-1} and gentamycin 0.1 mg ml^{-1}. The connective tissue capsule was cut (Fig. 1), allowing the neuronal cell bodies to pop out of the large glial cell that normally surrounds them[3]. For identification, the cells

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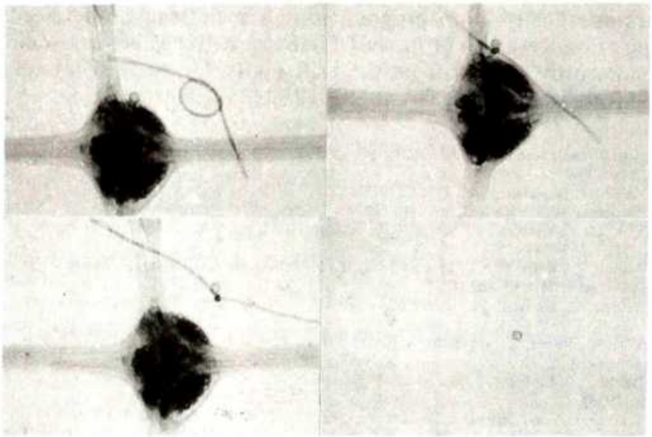

Fig. 1 Steps in the removal of an individual N (nociceptive) cell from a leech ganglion. The diameter of the N cell is about 60 μm.

were impaled with microelectrodes. However, this was not necessary for certain neurones which are unequivocally recognisable by their sizes and positions. Such cells include those used predominantly for the present experiments: the sensory cells that respond to pressure (P cells) and the large Retzius cells that cause mucus secretion. To isolate an identified cell a loop of fine nylon monofilament 13 μm in diameter was tied around its neck, pulled tight and drawn away from the ganglion (Fig. 1*a*). A few minutes later, after the cell had become sealed at the knot, it was freed by gently shaking the thread and transferred to a plastic tissue culture dish, coated with polylysine and collagen and containing approximately 2 ml of culture medium. Dishes were maintained at 20 °C, often without changing medium for 2 weeks.

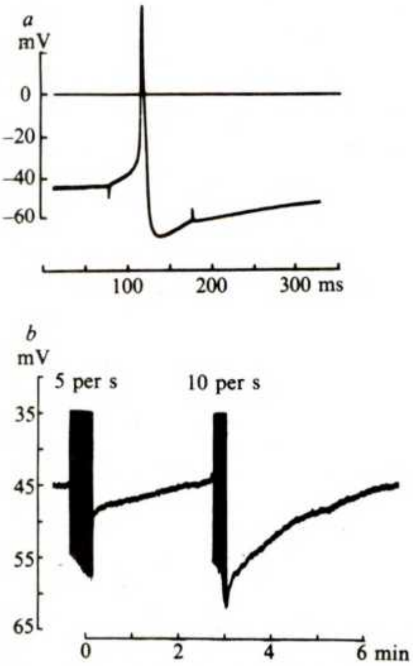

Fig. 2 *a*, Action potential from an N cell isolated for 21 days. The shape and undershoot are identical to those seen in N cells within an intact ganglion. *b*, Afterhyperpolarisation of a P sensory cell isolated for 24 days. Stimuli applied through the microelectrode produced impulses at 5 and 10 per s followed by a prolonged increase in membrane potential. In normal ganglia similar hyperpolarisation is caused by the activity of the Na–K pump and by an increase in K conductance.

For several weeks after removal from the ganglion, individual cells showed normal resting and action potentials. Figure 2*a* shows the action potential of a nociceptive (N) cell which had been kept isolated for 21 days. The shape of its action potential was indistinguishable from that of a normal N cell *in situ* and unlike that of any other type of cell in the ganglion[2]. Similarly, other cells, such as touch cells, pressure cells, and Retzius cells could also be identified unambiguously by electrical criteria and in particular by their characteristic action potentials. These results suggested that the intracellular concentrations of ions and membrane permeabilities were not grossly changed in culture. Further evidence was provided by stimulating cells repetitively. Fig. 2*b* shows the hyperpolarization following trains of impulses in a P sensory cell. The hyperpolarisation was comparable to that observed in normal P cells *in situ*, where it has been shown to arise from a coupled sodium pump and a calcium-dependent increase in potassium conductance[4].

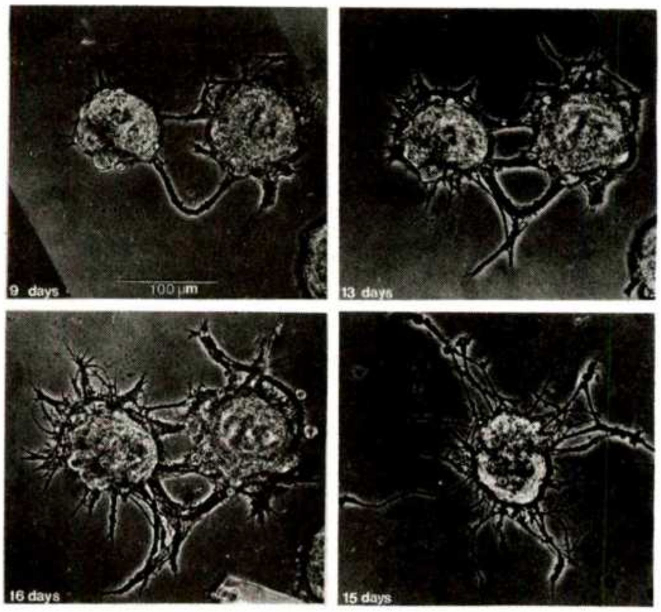

Fig. 3 Development of connections between a pair of Retzius cells over 16 days. Note the preferential distribution of processes running between the two cells and the relative paucity of processes running in other directions. The single neurone (a P cell) cultured on its own, shows more radial distribution of fibres.

After 5 to 7 days in culture, isolated cells began to sprout. Although leech neurones are monopolar *in vivo*, in culture a number of processes extended from the soma (Fig. 3). These processes had typical, filopodia-tipped growth cones, and tended to grow along each other, producing thickened, braided fascicles. Figure 3 shows the growth and development of neurites between two Retzius cells that were placed near one another. In this and other preparations studied over comparable times, bundles of processes were frequently observed passing between the cells, rather than at random. Single cells cultured alone sprouted, but usually without an obvious preferred direction (Fig. 3).

Cultured Retzius cells became electrically coupled and formed chemical synapses. The paired Retzius cells in each ganglion are electrically coupled to each other. When individual Retzius cells were dissected out and placed close to one another as in Fig. 3, or else in contact, they again became electrically coupled: current injected into one cell spread to its neighbour (Fig. 5). As in the animal, current passed equally well in both directions and considerable variation was seen in the degree of coupling, ranging from a minimal spread of current to nearly 1 : 1 coupling.

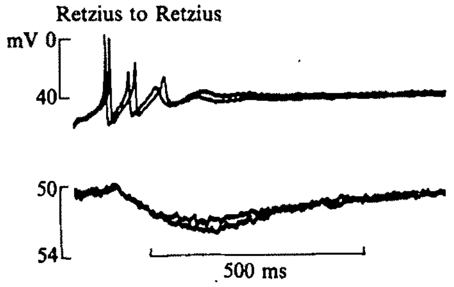

Fig. 4 Chemically mediated inhibitory potentials initiated at 22 days in one Retzius cell (lower trace) by impulses in its neighbour (upper trace). The electrical coupling between these cells was weak (see text). Impulses were initated at the end of hyperpolarising pulse (not shown).

While the development of coupling has not been studied in detail, in most cultures of closely opposed cells coupling first became obvious by about 10 days.

Retzius cells also formed chemical inhibitory synapses upon each other in culture. Figure 4 shows an example from a pair of Retzius cells in which impulses in one cell gave rise to hyperpolarizing potentials in the other with a variable latency. Such potentials were not obvious in cases where the degree of electrical coupling was strong and where the electronically conducted potential tended to obscure events occurring shortly after the spike. For similar reasons it is hard to test for inhibitory connections of this type between Retzius cells within the CNS. Towards the head of the leech, however, ganglia are occasionally found in which the Retzius cells are coupled only weakly and there it may be possible to demonstrate inhibitory connections if they are normally present.

A long-term goal of these experiments is to examine the specificity of connections formed between identified neurones in culture. So far we have obtained one clear example of preferential connections. Retzius cells were paired either with another Retzius cell or with a different neurone, the sensory P cell. In the ganglion the P cells are not electrically coupled to Retzius cells[6]. In five dishes kept from 12 to 24 days, 17 out of 24 pairs of Retzius cells became coupled. In contrast, none out of 22 Retzius–P pairs and none out of 8 P–P pairs became coupled (Fig. 5). In the leech, regenerating neurones reform their connections with a high degree of precision[5]. However, novel connections are occasionally found after lesions have been made to the CNS[7], and when ganglia are isolated in culture[5]. A similar relaxation of specificity may occur when individual cells are isolated and it would not be surprising if novel connections developed between cultured cells. For example, the inhibitory connections between Retzius cells may fall into this category.

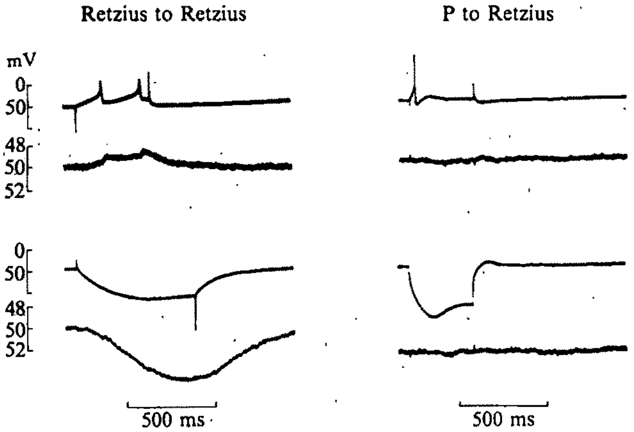

Fig. 5 Specificity of coupling at 24 days between paired Retzius cells (left) and paired Retzius P cells (right). Current spread from Retzius cell to Retzius cell but not from P cell to Retzius cell or vice versa. In this experiment the pairs of cells were in close apposition to one another in the same dish.

We conclude that adult leech neurones can survive well in culture without the large glial cells that normally surround them and can form certain specific electrical connections. The selectivity of connections formed under these conditions presumably depends on inherent properties of the cells rather than on factors such as timing, competition or the availability of sites for synapse formation on the target cell. Given the wide variety of identified cells available in the leech ganglion such experiments could provide a basis for examining in greater detail the preferences that a cell exhibits in relation to a number of different potential target cells.

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A physiological correlate of disuse-induced sprouting at the neuromuscular junction

W. D. Snider & G. L. Harris

Department of Physiology, School of Medicine, University of North Carolina, Chapel Hill, North Carolina 27514

Recent investigations have established that many of the normal properties of muscle fibres are maintained, at least in part, by muscle activity[1]. Thus, a fall in resting membrane potential[2,3], an increase in input resistance[4], and spread of acetylcholine receptors to extrajunctional sites[4–6] can all be induced by abolishing muscle activity and prevented by direct stimulation of denervated muscle fibres[7,8]. Muscle activity also exerts a trophic influence on the innervating motoneurones[9,10]; furthermore it may be a factor in the regulation of sprouting[11,12]. Brown and Ironton[11] found fine, "ultra-terminal sprouts" emanating from the endplates of muscles rendered inactive by chronic conduction block of the muscle nerve. Pestronk and Drachman[12] saw increased branching of the motor nerve terminal and a consequent increase in endplate size in similar conditions. If these sprouts at the endplates of inactive muscles were functional, one might expect more transmitter to be released in response to nerve stimulation[13]. We report here that both quantum content and spontaneous miniature endplate potential (m.e.p.p.) frequency are increased at the terminals of inactive (disused) muscles.

To effect disuse we used silicone cuffs impregnated with tetrodotoxin (TTX)[6]. TTX (1 mg) buffered with citric acid–sodium citrate (Sankyo) and 20 mg NaCl were dissolved in water; the solution was lyophilised and then mixed with 1.2 ml Silastic 382 (Dow Corning). Polymerising agent was added and the mixture was put in a glass tube (internal diameter, 4 mm) and hollowed out with a 17 gauge hypodermic needle. Female (except one) Sprague–Dawley rats (150–310 g) were anaesthetised with ether. The right or left sciatic nerve (experiments alternated sides) was exposed in the mid-thigh region, and a

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* Present address: Medical Service, Beth Isreal Hospital, 330 Brookline Ave, Boston, Massachusetts.

† To whom correspondence should be addressed.

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2–4-mm segment of the silicone cylinder was slit longitudinally and placed around the nerve. The efficacy of the block was assessed by checking the toe-spreading reflex which is invariably present when a normal animal is suspended by the tail; its absence is assumed to result from conduction block of the sciatic nerve[6]. The initial cuff abolished toe-spreading for 3–7 days. Rats were checked twice daily and a new cuff was implanted as soon as the reflex recovered. In this manner conduction block was maintained for a period of 8–9 days. Second cuffs were not usually as efficacious as the original, and sometimes as many as three changes were required. In some animals control cuffs, prepared as described above (including NaCl and citrate buffer) but without TTX, were implanted around the right or left sciatic nerve and left in place for 8–9 days. The toe-spreading reflex was not affected by the control cuff.

If conduction were blocked for 8–9 days, the animal was anaesthetised with Nembutal, and the soleus muscle, together with a length of muscle nerve, was excised from each leg. The muscles were pinned side by side in a lucite chamber superfused at room temperature by a solution of (mM): NaCl 136.8; KCl 5.0; $CaCl_2$ 1.0; $MgCl_2$ 8.0; NaH_2PO_4 1.0; $NaHCO_3$ 15.0, through which 95% O_2 plus 5% CO_2 was continuously bubbled. Neostigmine methylsulphate (10^{-6} M) was included in the solution to enhance the m.e.p.ps. Muscle fibres were impaled with conventional microelectrodes filled with 2.6 M KCl. Those fibres with a resting membrane potential of greater than 55 mV in which m.e.p.ps could be distinguished clearly were selected for determination of quantum content. On stimulating the muscle nerve at a frequency of 0.5 s^{-1}, ~200 endplate potentials (e.p.ps), then approximately 50 s of spontaneous m.e.p.ps were recorded. Recordings were taken alternately from disused and control muscles. Quantum content (m) was estimated by the method of failures[14] except for a few endplates where no failures occurred and m was calculated from the coefficient of variation of the e.p.p. amplitude distribution[14]. When both methods were applied for purposes of comparison (12 endplates), the m values agreed to within 15% of the average.

Table 1 Quantum contents at disused and control endplates

Expt	M_{TTX}	M_{con}	Ratio (M_{TTX}/M_{con})
1	2.3	1.8	1.3
2	2.9	1.8	1.6
3	3.3	1.8	1.8
4	3.4	3.5	1.0
5	4.9	4.2	1.2
6	3.2	2.4	1.3
7	2.2	1.4	1.6

M_{TTX} is the mean m of soleus endplates on the side where conduction of the sciatic nerve was chronically (8–9 days) blocked with TTX cuffs. M_{con} is the mean m of control endplates on the contralateral side. At least five fibres were sampled on each side.

Table 1 shows results obtained from seven animals. M_{TTX} represents the mean of m values determined at 5–7 soleus endplates on the disused side. M_{con} is the similarly determined mean from the contralateral control soleus of the same animal. The mean m of junctions from disused muscles was greater than that on the control side in six out of seven experiments, the average difference being 40%. These data were analysed with the two-tailed t test for matched pairs. The difference in mean quantum content between the disused and control sides was highly significant ($0.005 < P < 0.01$), and remained significant even when the two highest ratios (M_{TTX}/M_{con}) were excluded (expts 2, 3).

Table 2 presents data from five control animals, M_{cuff} being the mean m value of soleus endplates from the leg in which a control cuff (see above) had been placed. M_{con} is again from the contralateral control soleus. No significant difference in mean quantum content between the two sides was noted ($0.20 < P < 0.30$).

Table 2 Quantum contents from control animals

Expt	M_{cuff}	M_{con}	Ratio (M_{cuff}/M_{con})
1	1.5	1.5	1.0
2	1.7	2.2	0.8
3	1.3	1.3	1.0
4	2.5	2.4	1.0
5	1.3	1.6	0.8

M_{cuff} is the mean m of soleus endplates from the leg in which a control cuff had been placed. M_{con} is the mean m from the contralateral soleus.

At the amphibian neuromuscular junction, quantum content and spontaneous m.e.p.p. frequency (f) are positively correlated with endplate size[13]. If the terminal area is enlarged by sprouting, then one might expect f to increase as well as m. We made determinations of f from at least four disused and four control fibres in each of five experiments. After 8 days of disuse, f at junctions from disused muscles was higher than at contralateral control junctions in all five preparations. The mean values were respectively $1.2 \pm 1.0\ s^{-1}$ and $0.7 \pm 0.4\ s^{-1}$. With the matched pairs t test, f on the disused side was significantly higher ($0.02 < P < 0.05$). Animals prepared with control cuffs showed no significant differences in f between the cuff and contralateral sides. However, as was the case at soleus endplates in the mouse (S. T. Carbonetto and J. N. Weakly, personal communication), f and m were not significantly correlated in our data. One explanation may be that f and m vary less at mammalian than at amphibian endplates. For example, the coefficient of variation of m values from our control rat solei is 0.5, whereas in frog sartorius it is 1.2 (M. Kuno, S. A. Turkanis and J. N. Weakly, personal communication). The variability of f values from the two preparations is also quite different.

One might argue that the presence of the cuff could itself result in degeneration of some motor axons, and that partial denervation rather than disuse might account for these results. However, application of control cuffs did not result in any significant change in quantum content on the cuff side. Moreover, we did not observe fibrillation in any preparation, implying that most or all motor axons remained intact. Disuse itself has been reported to cause fibrillation[3,4], but work in the cat[9,15] agrees with our finding of none. We have no explanation for this discrepancy.

The present results indicate that at the neuromuscular junctions of chronically disused muscles, presynaptic terminals release increased amounts of transmitter in response to nerve stimulation and exhibit increased spontaneous release of acetylcholine quanta. These observations are consistent with the hypothesis that chronic disuse enlarges the functional endplate area, possibly as a result of increased terminal fibre branching and endplate length reported by Pestronk *et al.*[12]. Lømo and Rosenthal[4] have suggested that quantum content may be increased at the junctions of disused soleus muscles (slow twitch) but not those of disused extensor digitorum longus muscles (fast twitch). In the mouse, soleus endplates sprout more vigorously than peroneus tertius (fast twitch) endplates in response to disuse[11]. These findings suggest that fast and slow twitch muscles may differ in their ability to produce the signal for sprouting, or that there may be differences in the ability of their motor terminals to respond.

Disuse may affect transmitter release in other ways; for example, the membrane properties of presynaptic terminals may change, thereby affecting quantum content. While we cannot exclude this possibility, it is not clear how such changes in membrane properties would also increase spontaneous m.e.p.p. frequency. With respect to the signal that may trigger this increase in quantum content, it remains uncertain whether muscle inactivity or the disused terminals themselves are responsible. It is interesting in this regard that Gallego *et al.*[15] have reported an increase in synaptic efficacy at the disused Ia synapses of spinal motoneurones.

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GABA may be a neurotransmitter in the vertebrate peripheral nervous system

Kristján R. Jessen, Rhona Mirsky*, Marion E. Dennison & Geoffrey Burnstock

Department of Anatomy and Embryology, University College London, Gower Street, London WC1, UK

γ-Aminobutyric acid (GABA) is an inhibitory neurotransmitter in the peripheral nervous system of certain invertebrates and is thought to be a major transmitter in the vertebrate central nervous system[1–3]. In this report we present evidence that GABA may also be a neurotransmitter in the vertebrate peripheral autonomic nervous system. We have used light and electron microscopic autoradiography to analyse high-affinity uptake of ^{3}H-GABA into the myenteric plexus of the guinea pig taenia coli, both *in situ* and in a tissue culture preparation. In the isolated myenteric plexus, we have measured the specific activity of glutamic acid decarboxylase (GAD; EC 4.1.1.15), the enzyme responsible for conversion of glutamic acid to GABA in GABAergic neurones[4,5], and assessed the ability of this tissue to accumulate ^{3}H-GABA newly synthesised from ^{3}H-glutamic acid. Furthermore, we have measured the levels of endogenous GABA in strips of taenia coli containing the myenteric plexus.

For autoradiography, myenteric plexus from 1–8-day-old guinea pigs grown *in vitro* as described previously[6] was incubated in Krebs solution containing 2×10^{-8} M ^{3}H-GABA (1 μCi ml^{-1}, 66 Ci mmole^{-1}; [2,3-^{3}H]GABA, Radiochemical Centre) and 10^{-3} M β-alanine to block non-neuronal uptake of GABA[7]. After 20 min incubation at 20 °C, the samples were washed with Krebs solution, fixed in 4% glutaraldehyde in Krebs solution, washed with distilled water, air dried, dipped in Ilford L-4 or Kodak NTB2 emulsion and developed with Agfa Rhodinal developer after exposure for 10–14 days. Examination by light microscopy showed that although the density of silver grains was low over the non-neuronal cells and almost totally absent from most of the neurones, some neurones in the cultures were heavily labelled with grains (Figs 1, 2). Labelling was restricted to small ($\sim16\times17$ μm) to medium-sized neurones, the large neurones ($\sim40\times27$ μm) always being unlabelled. The grains seemed evenly distributed over the nucleus, cytoplasm and neuronal processes. The cell bodies were of various shapes and the labelled axons emanating from them could often be traced over long distances in the cultures, in some cases running together with unlabelled axons in thick axon bundles, but in others lying singly within the explants or in the outgrowth zones around them.

The heavy labelling was totally absent if 10^{-3} M *cis*-1,3-aminocyclohexane carboxylic acid (ACHC), an inhibitor of neuronal GABA uptake[8], was included in the incubation medium, thus confirming the morphological identification of the labelled cells as neurones. If the cultures were incubated without β-alanine but in the presence of 10^{-3} M ACHC a few cells within the explant were moderately labelled. This labelling was blocked by 10^{-3} M β-alanine and probably occurred over glial cells. Explant cultures of rat thoracic sympathetic ganglia showed no neuronal autoradiographic labelling in any of the above conditions.

These findings were extended by electron microscopic autoradiography of the myenteric plexus *in situ*. Taenia coli containing the myenteric plexus were dissected from 1-day to 3-month old guinea pigs and incubated with ^{3}H-GABA as

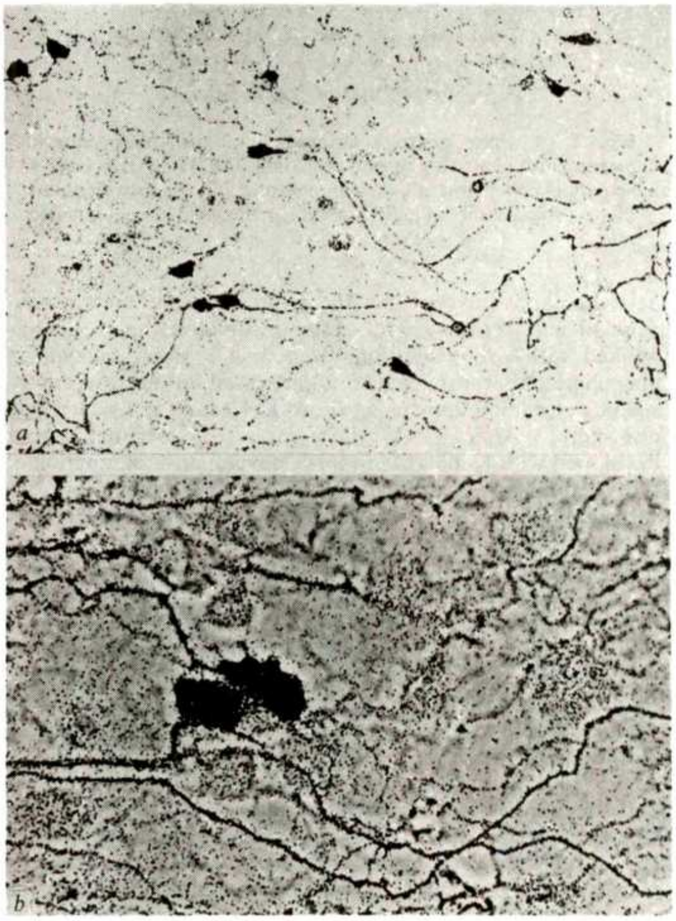

Fig. 1 Autoradiograph of myenteric plexus cultured for 10 d before incubation with ^{3}H-GABA. *a*, Bright field photograph showing the border between the explant (left hand side) and the outgrowth zone (right hand side). Radioactivity is concentrated over 10 neuronal cell bodies, most of which lie within the explant, and processes which are mainly seen in the outgrowth zone. The light background labelling, which is most noticeable within the explant, is over non-neuronal cells (×540). *b*, Phase contrast photograph of two heavily labelled neurones and their processes lying in the outgrowth zone. Note other labelled processes as well as light labelling concentrated over the nuclear areas of non-neuronal cells which form a continuous sheet underlying the neuronal elements of the outgrowth zone. The camera was focused on the autoradiographic grains (×720).

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* MRC Neuroimmunology Project, Department of Zoology, University College London, Gower Street, London WC1, UK.

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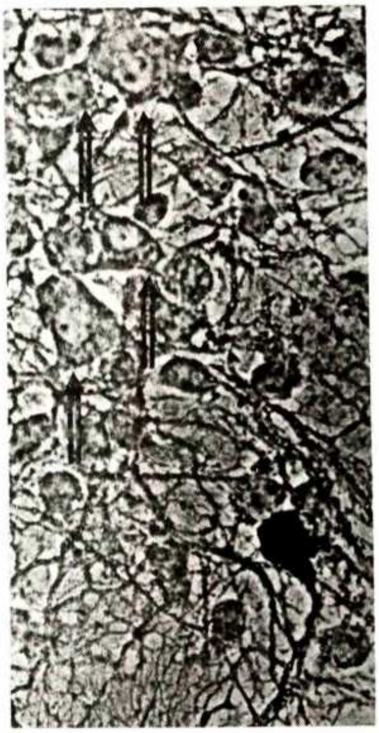

Fig. 2 A phase contrast autoradiograph of the myenteric plexus cultured for 12 d before incubation with ^{3}H-GABA. Radioactivity is concentrated over one neurone and the proximal part of its process; the other neurones in the field are unlabelled (arrows) (×630).

described above except that the incubation solution was oxygenated and kept at 37 °C. After incubation the tissue was washed with Krebs solution, fixed in 4% glutaraldehyde in phosphate buffer, osmicated and processed for electron microscopic autoradiography using Ilford L-4 emulsion as described elsewhere[9]. After 21–28 days exposure, specimens were developed with Kodak Microdol-X developer. Examination of thin sections through individual ganglia of the plexus and the surrounding smooth muscle showed three degrees of labelling (Fig. 3): heavy labelling over approximately 5% of the neurones in the ganglia and over some axons in nerve bundles running through the smooth muscle coat, light labelling over the glial cells of the ganglia and almost total absence of label over the smooth muscle and fibroblasts outside the ganglia and most of the neurones within the ganglia. The labelled neurones were small to medium sized, as far as could be judged by the examination of single thin sections, and were often elongated and found at the surface of the ganglia as illustrated in Fig. 3. The cytoplasm was fairly dark, being packed with free ribosomes and rough endoplasmic reticulum and the mitochondria were small and electron dense. They most closely resembled neuronal type 1 in the ultrastructural classification of myenteric neurones proposed by Cook and G.B.[10], the main discrepancy being the absence of accompanying satellite cells around the labelled neurones. The neuronal labelling, especially inside the ganglia, was greatly reduced if 10^{-3} M ACHC was present in the incubation medium. Thus, there is good agreement between the results obtained from the *in situ* and *in vitro* experiments both with respect to the distribution of label and the effects of uptake inhibitors.

Current evidence indicates that the only vertebrate neurones to possess a high-affinity uptake mechanism for GABA are GABAergic neurones, although it is uncertain whether all GABAergic neurones share this property[11,12]. This has recently received support from studies on ^{3}H-GABA uptake into spinal cord neurones in culture, where it was shown that no identifiable cholinergic neurones showed high-affinity GABA uptake[13]. The results above therefore raised the question as to whether there are GABAergic neurones in the myenteric plexus. If there are, the plexus should contain GAD and be able to accumulate newly synthesised GABA. Furthermore, it should contain endogenous GABA, and pharmacological actions of GABA in the gut ought to be detectable.

We measured the specific activity of GAD by formation of labelled GABA from labelled glutamic acid as described by others[14], in three separate experiments. The plexus from the taenia coli of 1–8-day-old guinea pigs, dissected completely free of smooth muscle as described previously[6], was homogenised in ice-cold distilled water and incubated at 37 °C for 15, 30 and 60 min in a solution containing the following components (final concentrations): potassium phosphate, *p*H 6.5, 0.1 M; dithiothreitol, 0.5 mM; pyridoxal phosphate, 0.5 mM; sodium L-glutamate, 25 mM; L-[*G*-^{3}H]glutamic acid, 500 μCi ml^{-1}, electrophoretically purified (specific activity 31 Ci mmol^{-1}, Radiochemical Centre). The total amount of protein in each experiment was 30–50 μg. This was divided into six samples and the levels of ^{3}H-GABA obtained were 1.5 to 5 times higher than the assay blanks. Significant levels of GAD were detectable, the specific activity being 2.18 ± 1.2 μmol per h per 0.1 g protein.

Neurones can not only synthesise but also accumulate in high concentrations the transmitter they use. We therefore investigated whether accumulation of GABA in intact cells of the myenteric plexus could be detected following incubation of the tissue with radiolabelled glutamic acid[15]. For comparison, we also studied slices of rat cerebellar cortex, which contains GABAergic neurones[4,5], and rat superior cervical ganglion,

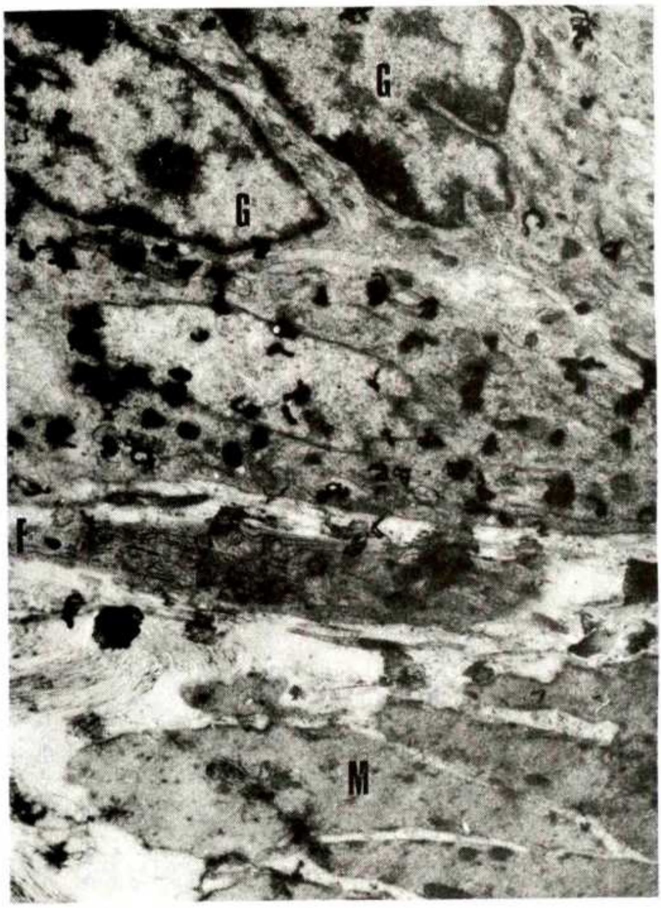

Fig. 3 Electron microscopic autoradiograph of the myenteric plexus incubated *in situ* with ^{3}H-GABA. The micrograph shows the edge of a ganglion with two slightly labelled glial cells (G), identifiable by the morphology of the nucleus[31], a heavily labelled neurone, a fibroblast (F) in the interstitial space between the ganglion and the surrounding muscle, and a part of the muscle layer (M), all of which are virtually unlabelled (×13,000).

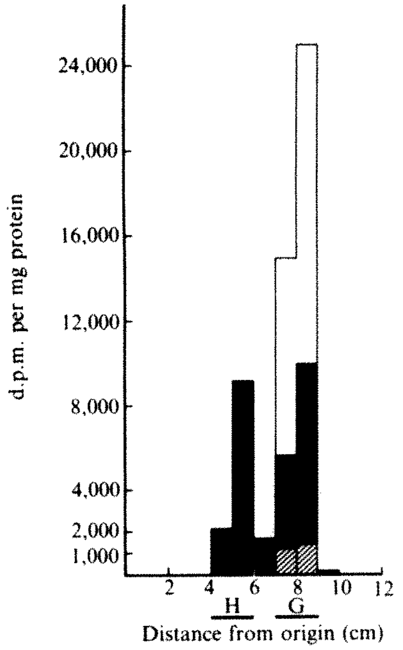

Fig. 4 Paper chromatography of ^{3}H-GABA and ^{3}H-homocarnosine synthesised from ^{3}H-glutamic acid in myenteric plexus (solid columns), cerebellum (open columns) and superior cervical ganglion (cross-hatched columns). The figure shows the distribution of radioactivity expressed as d.p.m. per mg protein based on results from three representative experiments, one for each tissue. The bars mark the position of non-radioactive homocarnosine (H) and GABA (G) on the chromatogram. The tissues were incubated in two changes of Eagle's minimum essential medium containing 6 g l^{-1} glucose and 10% fetal calf serum, for 40 min. The tissues were then placed in the same medium containing 4×10^{-6} M ^{3}H-glutamic acid, 100 μCi $mmol^{-1}$ (23 Ci $mmol^{-1}$; Radiochemical Centre) for 17 h at 37 °C. In one experiment electrophoretically purified ^{3}H-glutamic acid was used. After the incubation the samples were washed in two changes of phosphate-buffered saline, containing 150 mg l^{-1} non-radioactive glutamic acid, for 40 min and homogenised in 20 μl of ice-cold distilled water. A 5-μl sample of homogenate was withdrawn for protein estimation[32] and 20 μl of 0.47 M formic acid, 1.4 M acetic acid containing 1 mg ml^{-1} of glutamic acid, GABA and in some cases homocarnosine was added to the remainder of the sample, which was frozen and thawed three times before being subjected to high-voltage paper electrophoresis[33]. The paper was dried and ascending chromatography in *n*-butanol/acetic acid/water (60:15:25)[17] was carried out at right angles to the direction of electrophoresis. The figure shows the distribution of radioactivity resulting from chromatography of that section of the paper to which non-radioactive GABA ran in the electrophoresis. In other experiments not shown here, *t*-butyl alcohol/methyl ethyl ketone/water/formic acid (8:6:5:1)[21] was used as the solvent system. The amino compounds were visualised with ninhydrin spray, the paper cut into strips 1 cm long and 3.2 cm wide, the radioactivity eluted with 0.1 M HCl, followed by 4.5 ml of Aquasol (NEN) and counted in a scintillation counter.

where there is no evidence of GABAergic neurones[16,17]. Isolated myenteric plexus, 400-μm thick slices of cerebellar cortex and desheathed superior cervical ganglia from 4–6-week-old rats were incubated with ^{3}H-glutamic acid for 17 h at 37 °C. They were then homogenised and analysed for the presence of radioactive products of ^{3}H-glutamic acid by electrophoresis, chromatography in two different systems and subsequent scintillation counting. The results are shown in Fig. 4. The myenteric plexus, cerebellum and superior cervical ganglion all converted ^{3}H-glutamic acid to ^{3}H-GABA. The myenteric plexus contained 20,100±3,000 d.p.m. or 4.0×10^{-13} mol of ^{3}H-GABA per mg protein, the cerebellum contained 41,500±11,000 d.p.m. or 8.0×10^{-13} mol of ^{3}H-GABA per mg protein and the superior cervical ganglion contained 4,800±1,300 d.p.m. or 9.6×10^{-14} mol per mg protein. Each value is an average of four experiments, all using the *n*-butanol/acetic acid/water chromatography system as described in Fig. 4 legend. In medium incubated without tissue no conversion was detectable. Thus, in these incubation conditions both the myenteric plexus and cerebellum contained significantly greater quantities of ^{3}H-GABA than the superior cervical ganglion, where synthesis and accumulation of ^{3}H-GABA apparently takes place in glial cells only[16–19].

In the case of myenteric plexus about 40% of the radioactivity running with non-radioactive GABA in the high-voltage electrophoresis separated from it in subsequent chromatography, forming a second peak of radioactivity. We found that homocarnosine (γ-aminobutyryl-L-histidine), a dipeptide which can be formed from GABA and histidine[20], also ran in the same position as GABA on the high-voltage electrophoresis and separated from it in two separate chromatography systems to run in an identical position to the second radioactive peak. We therefore conclude that this peak, which amounted to 13,400±2,200 d.p.m., is homocarnosine. The absence of ^{3}H-homocarnosine from the rat cerebellar cortex and superior cervical ganglion is not merely due to a species difference in GABA metabolism, as no ^{3}H-homocarnosine was detected in similar experiments with guinea pig superior cervical ganglia and cerebellar cortex, where similar levels of ^{3}H-GABA to those in the rat tissues were accumulated. We know of no other report on the synthesis or occurrence of homocarnosine in tissues other than brain. There it is present in low amounts but with heterogeneous distribution which, contrary to earlier beliefs, does not parallel that of GABA[21]. Homocarnosine is a potent anti-seizure agent[22,23], causes hyper-excitability and shortens barbiturate sleeping time[24] when injected into cerebral ventricles, and has been postulated to be a central neurotransmitter[25]. Considerable evidence has also accumulated that carnosine, a related dipeptide (β-alanyl-L-histidine), may be a neurotransmitter in the olfactory bulb[26].

When measuring endogenous GABA levels, the rapid and significant accumulation of GABA which takes place after the death of the animal, must be taken into account[27]. Thus, GABA levels in the myenteric plexus would probably increase substantially during the time it takes to dissect it from the gut wall. To assess whether the plexus contained GABA we therefore compared GABA levels in strips of taenias containing the plexus to those in muscle (gastrocnemius) which does not contain the plexus, both tissues being removed from living anaesthetised guinea pigs and frozen immediately in liquid N_2. The samples were then extracted twice with 80% ethanol, freeze dried and analysed for the presence of GABA with an automated amino acid analyser with a fluorimeter attached[27]. Three experiments gave a value of 17.5±3.1 nmol GABA per g wet weight of tissue for taenia coli compared with 5.7±0.1 nmol GABA per g wet weight of tissue for muscle. The difference between the two tissues can probably be attributed to the myenteric plexus, in which case, if it were possible to measure endogenous GABA levels in the myenteric plexus alone, the values would be considerably higher than those reported here, as in the taenia the plexus is embedded in a large mass of muscle.

Few studies have been carried out on the action of GABA in the gastrointestinal tract. However, Hobbiger reported that 10^{-5} M GABA blocked the peristaltic reflex of the guinea pig ileum although it did not seem to act directly on smooth muscle. A variety of other effects of GABA on gut preparations have led to the suggestion that it may serve as a neurotransmitter[28–30].

Thus, we have shown that a small population of neurones in the myenteric plexus possesses a high-affinity uptake mechanism for GABA. These neurones are small to medium sized and share several common ultrastructural characteristics. Furthermore, the myenteric plexus contains GAD and endogenous GABA and can synthesise and accumulate ^{3}H-GABA and ^{3}H-homocarnosine from ^{3}H-glutamic acid. The complexities of GABA metabolism and compartmentation are such that these results should be interpreted with caution. However, in conjunction with the pharmacological evidence that GABA affects intestinal peristalsis, the results suggest that it may have a role in neuronal transmission in the gut.

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Expression of a liver-specific function by mouse fibroblast nuclei transplanted into rat hepatoma cytoplasts

Leah Ann Lipsich, Joseph R. Kates & Joseph J. Lucas

Department of Microbiology, Health Sciences Center, State University of New York at Stony Brook, Stony Brook, New York 11794

Data from many somatic cell fusion experiments support the notion that the cytoplasm of eukaryotic cells contains elements which can alter gene expression and replication[1]. More definitive evidence is perhaps lacking because the cell hybrids studied were complex cells comprising mixed genomes, frequently undergoing chromosomal losses and other aberrations, within mixed cytoplasms. Such problems have been alleviated by the development of the techniques of cybridisation, red cell-mediated microinjection and nuclear transplantation[2]. Using this last technique, we showed that up to 40% of a monolayer culture of cytoplasts could be renucleated to form whole viable cells[3,4]. Because of the high efficiency of fusion and the purity of cytoplast and karyoplast cultures used, large quantities of true cytoplasmic–nuclear hybrid cells suitable for immediate morphological and biochemical analysis could be formed. Here we describe the application of the technique to an investigation of the effects of rat liver cytoplasm on gene expression by mouse fibroblast nuclei. We show that the synthesis of mouse tryosine amino transferase (TAT)—a liver-specific enzyme never detected in the fibroblast cell line was induced in hybrids constructed by transplantation of mouse (A9) fibroblast nuclei into rat liver (HTC) cytoplasts. Furthermore, as occurs in the rat cell line, its activity was modulated by addition of the synthetic corticosteroid, dexamethasone. Clearly, this hybrid cell system will be useful in the further characterisation and eventual isolation of the newly identified regulatory substance.

First, hybrid cells were formed and identified using our previously described techniques[3]. Briefly, cytoplasts were prepared from the HTC rat hepatoma cell line, which can be routinely enucleated with a >99% efficiency. Monolayer cultures of cytoplasts were treated with inactivated Sendai virus, after which excess unabsorbed virus was removed and replaced by a suspension of karyoplasts prepared from the A9 mouse fibroblast cell line. After fusion, the dishes were washed vigorously to remove virus and excess unfused karyoplasts. Freshly prepared karyoplasts cannot adhere to a surface; only those fused to the cytoplast monolayer will remain associated with the dish. Approximately 20% of the HTC cytoplasts were routinely renucleated, with the value ranging from 10 to 30% in over 30 separate experiments. In cultures in which karyoplasts were layered over cytoplasts in the absence of virus, fewer than 1% of bodies contained nuclei, these cells being that small fraction of HTC cells not originally encleated during centrifugation in medium containing cytochalasin B (see ref. 3 and below). Figure 1*a* shows an A9 nucleus–HTC cytoplast hybrid cell culture 18 h after fusion. By this time most cytoplasts not receiving nuclei have died and disintegrated. On the second day after fusion, medium containing 8-azaguanine was placed on the cells. The A9 nucleus does not code for the enzyme hypoxathine–guanine phosphoribosyl transferase (HGPRT); thus, hybrids should also be resistant to the toxic analogue. As shown in Fig. 1*b*, the culture continued to grow (in fact, these cells were subcultured several times during the 2 weeks of growth), whereas a culture of whole HTC cells died within this time (Fig. 1*c*). The addition of azaguanine was delayed for 2 days to permit degradation of HGPRT contributed to the hybrids by the HTC cytoplasm[3].

It was established, therefore, that the A9–HTC hybrids were viable and that they retained a genetic marker of the mouse nuclear donor cell. Furthermore, use of azaguanine permitted elimination of the small background of whole HTC cells originally contaminating the cytoplast cultures. As further proof that the cells contained mouse nuclei, karyotyping[5] was carried out. Distinction between the two nuclei is possible as HTC cells contain several matacentric marker chromosomes[6] whereas the mouse cells contain primarily telocentric chromosomes. Metaphase chromosome spreads were prepared from A9, HTC and hybrid cells (grown for 2 weeks) and scored for the presence of metacentric chromosomes. 100% of spreads from HTC cells contained metacentric chromosomes whereas 8% of spreads from A9 cells contained chromosomes that were scored as metacentric. Of all spreads examined from hybrid cells, only 4% contained chromosomes appearing metacentric. Thus, these results suggested that the constructed cells contained mouse, not rat, nuclei. Note also that hybrids could be formed, using similar techniques, by fusion of A9 cytoplasts and HTC karyoplasts. As expected, such hybrids were killed by 8-azaguanine.

With techniques for the routine preparation of hybrids now available, we assayed the cells for liver-specific functions. First,

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Table 1 Histochemical assay for tyrosine amino transferase

Cells	Standard assay	Assay after heat treatment
Mouse fibroblast A9	−	−
Rat hepatoma HTC	+ +	−
A9 nucleus–HTC cytoplasm hybrids at		
24 h	+	+
48 h	+	+
72 h	+	+
96 h	+	+

Hybrids were constructed as described in Fig. 1. All cells (hybrids, A9s and HTCs) were treated for 24 h with 10^{-5} M dexamethasone before assay. The standard assay was a modification of the procedure described by Thompson and Tomkins[7]. Briefly, monolayers of cells were washed three times with buffer (1 M potassium phosphate, *p*H 7.6, 0.2 mM pyridoxal phosphate, 0.5 mM α-ketoglutarate) and allowed to air dry for 1 h at room temperature. The enzyme was then assayed as described[7]. For heat treatment, the monolayers were first heated to 75°C for 10 min and then assayed as above. The reaction product was visualised using a Zeiss research microscope. − Indicates that no reaction product could be seen in the cells; they appeared clear; + + indicates an intense purple reaction product in the cells; + indicates a reaction which is less intense than the HTC cultures but still distinctly purple.

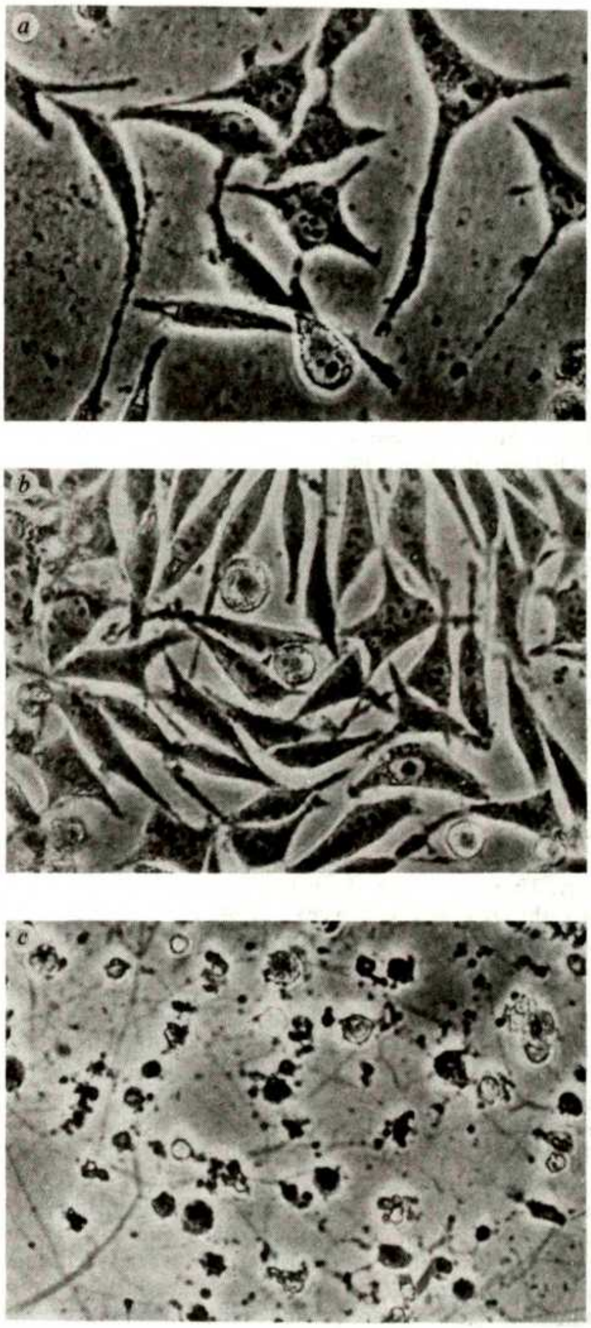

Fig. 1 Hybrid cells constructed by transplantation of mouse fibroblast A9 nuclei into rat hepatoma HTC cytoplasts. *a*, A culture at 18 h after formation; *b*, the same culture after 2 weeks of growth in azaguanine (after several subcultivations); *c*, HTC cells after 1 week in azaguanine (without subcultivation). Magnification, ×150. The A9 parent cell line was maintained in monolayers in Dulbecco's modified Eagle's medium supplemented with 10% calf serum; the HTC parent line was grown in Waymouth's MAB 87/3 supplemented with 10% fetal calf serum. Previously described techniques were adapted for cytochalasin B-induced enucleation of the A9 and HTC cell lines. Cytoplasts and karyoplasts were then fused by Sendai virus-mediated fusion as described elsewhere[3,12].

monolayer cultures were formed by transplantation of mouse fibroblast nuclei into rat hepatoma cytoplasts. At 0, 24, 48 and 72 h after fusion, a culture was incubated in dexamethasone for a further 24 h and then assayed histochemically[7] for TAT activity. Cultures of whole A9 and HTC cells were similarly treated. As noted in Table 1, HTC cells exhibited a deep purple pigmentation but A9 cells were entirely clear. Hybrid cells, at all times measured, showed an intermediate level of staining. To determine whether the reaction product seen in the hybrids was due to the mouse liver form of the enzyme, and was not merely residual rat enzyme or a non-inducible mouse pseudoenzyme, heat inactivation experiments were carried out. Both the rat TAT and mouse pseudoenzymes are considerably more thermolabile than the corresponding mouse liver enzyme. If the former enzymes are heated carefully to 75 °C for 10 min, activity is markedly reduced; mouse liver TAT, however, retains much of its activity[8]. Therefore, monolayer cultures of hybrids, A9 and HTC cells were first heated to 75 °C for 10 min and then assayed histochemically. As shown in Table 1, the appearance of reaction product in HTC cells was completely eliminated by heating. The intermediate level of response exhibited by hybrid cells was apparently unaffected. These results were obtained six times using hybrid cells constructed in separate experiments. In all the experiments presented in Table 1, cultures were treated with dexamethasone to maximise the intensity of the stain. However, in control cultures not treated with the steroid, A9 cells again exhibited no reaction product, HTC cells showed a distinctly purple but much less intense colour than induced cells, and the hybrids exhibited a barely detectable stain. It therefore seemed that the mouse enzyme produced was not only heat stable but also inducible. The magnitude of the induction was quantitated in later cultures as described below.

The observations were confirmed and quantitated using an *in vitro* biochemical assay for enzyme activity[9,10]. In these experiments, carried out on 10 separate occasions, cells were assayed at various times during the second and sixth week after fusion. As summarised in Table 2, the hybrids formed by transplantation of A9 nuclei into HTC cytoplasts exhibited levels of enzyme activity greater than 25% of that shown by induced HTC cells. Note that (1) the hybrids could be grown in the presence of azaguanine, and that (2) TAT-producing cultures were confirmed as mouse cells by karyotyping, as described above. Furthermore, the indication that the enzyme made was indeed the steroid-inducible liver form of the enzyme was confirmed by experiments showing that the enzyme produced by hybrid cells was precipitable by antiserum against purified rat liver TAT. As shown previously[8], such antiserum effectively precipitates both rat and mouse liver TATs, but not non-inducible mouse pseudoenzyme.

All the results in Table 2 were obtained from cultures between the second and sixth weeks after fusion. At earlier times it was difficult to obtain the number of hybrids needed for this biochemical assay. However, we considered it essential to assay

Table 2 Enzymatic assay of tyrosine amino transferase

Cells	Milliunits enzyme activity per 5×10^6 cells −Dexamethasone	+Dexamethasone
Mouse fibroblast A9*	1.30	1.24
Rat hepatoma HTC*	6.80	38.69
A9 nucleus–HTC cytoplasm hybrids†	3.77	10.65
HTC nucleus–A9 cytoplasm hybrids‡	Not done	45.08

Hybrids were constructed as described in Fig. 1. Assays were carried out with and without a 24-h treatment with dexamethasone (10^{-5} M). Cytoplasmic extracts prepared from cells were assayed for TAT by a modification of the Diamondstone assay[9], as described by Granner and Tomkins[10]. Assays were carried out on hybrids at various times during the second and sixth weeks after fusion.

* Data represent means of 10 separate assays.

† Data represent means obtained from 10 separate fusion experiments.

‡ Datum represents mean obtained from three separate fusion experiments.

early hybrids by the more accurate, quantitative *in vitro* assay. Therefore, 15 separate cultures of HTC cytoplasts were fused to A9 karyoplasts and pooled at 24 h after preparation. (Because of the ratio of karyoplasts to cytoplasts required to obtain maximum fusion, it was necessary to prepare A9 karyoplasts by enucleation of 128 cultures.) The steroid-induced level of activity in HTC cells was 28.4 mU per 5×10^6 cells, whereas the activity in 24-h hybrid cells was 7.6 mU per 5×10^6 cells. Three-week-old hybrids assayed at the same time yielded 6.4 mU of activity. Thus, the levels of enzyme produced were apparently similar throughout the 6 weeks of study. Furthermore, the enzyme present in these early hybrids was shown to be the heat-stable mouse liver form of the enzyme: after heat treatment which reduced the rat HTC enzyme activity to background levels (<1 mU per 5×10^6 cells), the hybrid cell enzyme retained, as predicted, 30% of its activity (see ref. 8). Also noted in Table 2 are observations that hybrids constructed by transplantation of HTC nuclei into A9 cytoplasts produced enzyme at levels comparable to those of the parent rat liver cell.

The data presented here were obtained from cells assayed up to 6 weeks after fusion. In all 10 experiments in which A9 nuclei were transplanted into HTC cytoplasts, however, the hybrids abruptly ceased making TAT between 6 and 8 weeks after fusion. The loss of activity was correlated with a transient change in morphology and death of many cells, after which the cultures continued to grow, apparently indefinitely, without detectable TAT activity. The period during which TAT was expressed represented some 75–100 cell generations. This would be sufficient to result in a greater than 10^{20}-fold dilution of cytoplasmic elements originally present in the hepatoma cytoplast donor cell.

This system, exhibiting the induction and modulation of an activity not normally expressed by the nuclear parent, is ideal for further analysis of the cytoplasmic elements capable of altering gene expression. Moreover, the induced programme of expression is apparently specific. Thus, the hybrids did not synthesise albumin, a liver-specific function not retained by HTC cells grown in culture (data not shown). We are now attempting to induce the synthesis of TAT in A9 cells by microinjection of components prepared from the cytoplasm of liver cells, using techniques we previously used to microinject cytoplasmic elements capable of inducing DNA synthesis in dormant nuclei[11]. If successful, such experiments would provide the basis for an assay system useful for the purification of the inducing substance.

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Isolation by sucrose-density fractionation and cultivation *in vitro* of actinomycetes from nitrogen-fixing root nodules

Dwight Baker & John G. Torrey

Cabot Foundation for Botanical Research, Harvard University,
Petersham, Massachusetts 01366

George H. Kidd*

The Biological Laboratories, Harvard University, 16 Divinity Avenue,
Cambridge, Massachusetts 02138

The fixation of atmospheric nitrogen by actinomycete-nodulated, woody dicotyledonous plants represents a substantial contribution to the global nitrogen cycle[1], contributing especially to forested areas, wetlands, fields and disturbed sites of temperate and tropical regions[2]. The study of these nitrogen-fixing symbioses has been hampered by difficulties in isolating and culturing the microsymbionts *in vitro*. Recently, root nodule actinomycetes have been isolated by microdissection techniques and cultured *in vitro*[3,4]. We report here the isolation and culture for the first time of the actinomycetes associated with root nodules of *Elaeagnus umbellata* (Elaeagnaceae) and *Alnus viridis* ssp. *crispa* (Betulaceae) (shown in Fig. 1*a* and *b*, respectively), using a technique radically different from those used previously. For separating actinomycetes from root nodules, we have used the simple fractionation technique of sucrose-density sedimentation.

Root nodules (0.25 g fresh weight) from plants of *E. umbellata* Thunb., growing in nitrogen-free one-quarter-strength Hoagland's water culture solution[5], were surface-sterilised for 15 min in 50 ml of a 30% (v/v) solution of Clorox (5.25% NaOCl) to which one drop of Tween 20 had been added as a surfactant. The nodules were washed twice in sterile distilled water and then crushed in a sterile mortar and pestle with a small amount of sterile distilled water. The resultant slurry was filtered serially through Miracloth (Chicopee Mills) and glass wool and diluted to 10 ml with sterile distilled water.

Cellulose-nitrate centrifuge tubes were sterilised with 95% ethanol and permitted to air dry. Using heat-sterilised sucrose solutions, discontinuous density gradients were constructed of a 3-ml cushion of 60% (w/v) sucrose, a 3-ml layer of 45% (w/v) sucrose and a 6-ml layer of 30% (w/v) sucrose. Two ml of the filtered nodule suspension were layered onto each gradient, and the gradients centrifuged at 100,000*g* for 3 h at 4 °C in a Beckman L2-65B ultracentrifuge equipped with a SW-40 rotor. The gradients were fractionated by aseptically piercing the bottoms of the tubes with a 25-gauge needle and collecting eight

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* Present address: Battelle-Columbus Laboratories, 505 King Avenue, Columbus, Ohio 43201.

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1-ml fractions. The sucrose-density fractions were used to inoculate agar plates of nutrient medium containing yeast extract 0.5%, dextrose 1.0%, casamino acids 0.5%, H_3BO_3 1.5 mg l^{-1}, $ZnSO_4 \cdot 7H_2O$ 1.5 mg l^{-1}, $MnSO_4 \cdot 2H_2O$ 4.5 mg l^{-1}, $NaMoO_4 \cdot 2H_2O$ 0.25 mg l^{-1}, $CuSO_4 \cdot 5H_2O$ 0.04 mg l^{-1}, vitamin B_{12} 1.6 mg l^{-1} and Difco agar 0.8%. The plates were sealed with Parafilm and incubated at 28 °C.

Colonies of a finely filamentous actinomycete appeared on the inoculation plates after approximately 3–5 weeks. The frequency of this organism in the sucrose-density fractions as demonstrated by its growth on the isolation plates was greatest in fraction 4, corresponding to the interface between the 60% and 45% sucrose layers. At 8 weeks this '*Elaeagnus* isolate' was subcultured to agar and liquid media for further observation and characterisation. The isolate grows in dense colonies composed of branching septate filaments which have diameters of approximately 0.3 μm (Fig. 1*a*). Sporangia, attached by a thickened sporangiophore, are produced terminally on the submerged filaments. Spores within the sporangia are roughly 1 μm in diameter and spherical to oblong. The *Elaeagnus* isolate will grow on the surface of agar, but true aerial mycelia or aerial sporangia are not formed. Colonies produce variable amounts of blood-red pigment that diffuses into the culture medium. We have designated the isolate *Frankia* sp. EuI1 until the uncertainty of specific epithets[6] can be resolved.

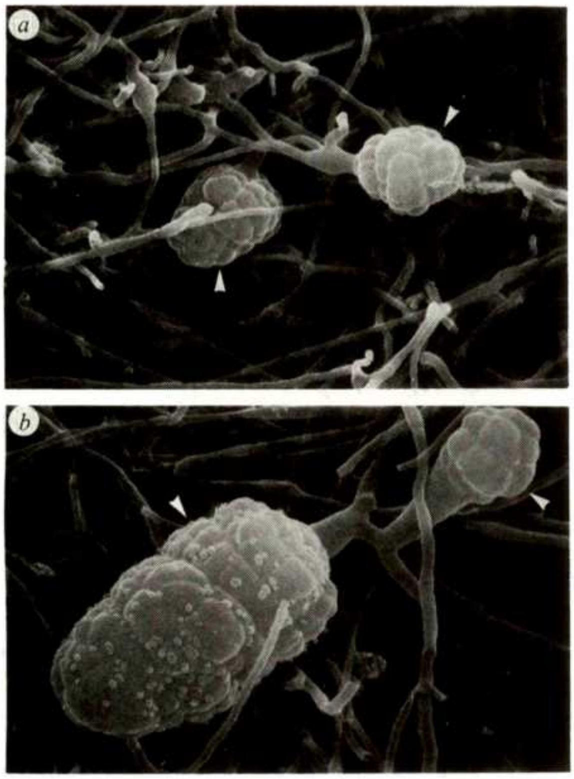

Fig. 1 Scanning electron micrographs of *in vitro* cultured actinomycete microsymbionts, ×3,550 (specimens fixed in glutaraldehyde, dehydrated in ethanol, critical-point dried with liquid CO_2 and sputter-coated with gold–palladium). *a*, Actinomycete isolated from root nodules of *E. umbellata*. Sporangia (arrows) contain many small spores. *b*, Actinomycete isolated from root nodules of *A. viridis* ssp. *crispa*. Arrows indicate sporangia.

Infectivity tests were carried out to confirm that the *Elaeagnus* isolate was in fact a symbiotic endophyte. *Elaeagnus* seedlings, germinated and grown in moist sand for 4 weeks, were transplanted into 400 ml standing water cultures supplied with Hoagland's nitrogen-free solution. The plants were grown in a controlled-environment chamber under a light–dark cycle for 16 h and 8 h, respectively, and a day–night temperature cycle of 27 °C and 21 °C, respectively. After 1 week in the nitrogen-free solution, the plants were inoculated with a small amount of washed, homogenised *Elaeagnus* isolate from 4–6-week old broth cultures. Control plants were either uninoculated or inoculated with a crushed nodule inoculum (0.3 g ground *Elaeagnus* nodules in 5 ml water).

After 4 weeks, young developing nodules were evident on many inoculated plants. Uninoculated control plants bore no nodules. At 8 weeks, plants inoculated with a crushed-nodule inoculum were beginning to recover from nitrogen starvation whereas those inoculated with the *Elaeagnus* isolate showed no signs of recovery. At this time the number of nodules per plant was counted and routine acetylene reduction tests[7] were carried out on the observed nodules to determine nitrogenase activity (Table 1). Nodules initiated by the crushed nodule inoculum exhibited acetylene reduction rates comparable to previous reduction values for other actinomycete-nodulated plant species[8]. Nodules initiated by the *Elaeagnus* isolate showed no measurable acetylene-reduction activity and were clearly ineffective.

Figure 2*a* shows the endophyte within host cells of a plant inoculated with a crushed nodule suspension. Figure 2*b* shows the endophyte within nodule cells of a plant inoculated with *Frankia* sp. EuI1. Typical vesicles seen in the nodules initiated by a crushed nodule inoculum (Fig. 2*a*) were absent in the nodules initiated by the *Elaeagnus* isolate (Fig. 2*b*).

In view of the above successful isolation, described sucrose-density fractionation procedure was repeated step-by-step using 0.5 g (fresh weight) of nodules from *Alnus viridis* ssp. *crispa* (Aiton) Turrill collected near Atikokan, Ontario, Canada. After 3 weeks of incubation, a finely filamentous actinomycete was observed growing on the agar plates inoculated with sucrose-gradient fraction 4.

Like the *Elaeagnus* isolate, the '*Alnus* isolate' grows in radial colonies composed of branching septate filaments bearing sporangia, attached by a thickened sporangiophore (Fig. 1*b*). However, unlike species EuI1, the *Alnus* isolate grows more diffusely, will not grow on the surface of agar and does not produce any pigments. Filament diameter of the *Alnus* isolate is approximately 1 μm but sporangia are much larger (20–60 μm, Fig. 1*b*) than those of the previously isolated nodule actinomycetes. Spores are slightly smaller than 1 μm. We have designated this isolate *Frankia* sp. AvcI1.

Infectivity tests were carried out using the *Alnus* isolate or a crushed nodule suspension (0.7 g ground *Alnus* nodules in 5 ml distilled water). After 10 d young developing nodules were evident on inoculated plants. Uninoculated control plants bore no nodules. At 4 weeks the number of nodules per plant was counted and acetylene reduction assays were carried out on the observed nodules (Table 1). Acetylene-reduction values of the isolate-inoculated plants were greater than those of plants inoculated with nodule suspensions. When endophyte morphology was observed by scanning electron microscopy, the

Table 1 Nodulation and acetylene reduction values for seedlings of *Elaeagnus* and *Alnus*

Treatment	No. of test plants	Plants nodulated (%)	Nodules per plant*	Acetylene reduction†
Elaeagnus, uninoculated	6	0	0	—
Elaeagnus, nodule suspension	15	87	9.9	4.7–5.0
Elaeagnus, *Frankia* sp. EuI1	30	33	6.2	0
Alnus, uninoculated	6	0	0	—
Alnus, nodule suspension	15	20	1.3	Negligible
Alnus, *Frankia* sp. AvcI1	30	100	22.7	0.46–2.68

* Values are expressed as an average computed from those plants bearing root nodules and do not include unnodulated plants.

† Values are expresed as μM ethylene per g freshweight per h; uninoculated control plants were no measured for reductin activities.

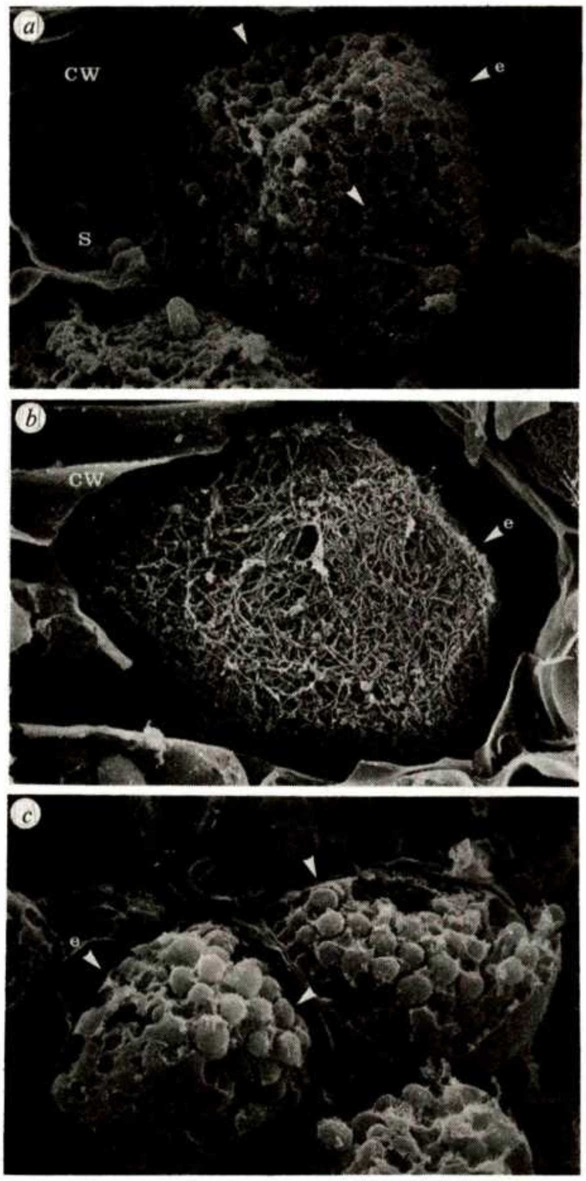

Fig. 2 Scanning electron micrographs of infected cells of host plant root nodules, ×1,100 (samples prepared as in Fig. 1); e, endophyte cluster; cw, host cell wall; s, starch grain. *a*, Actinomycete endophyte within cells of *Elaeagnus* inoculated with a crushed nodule suspension. Vesicles (small arrows) are borne terminally on mycelial branches. *b*, Actinomycete endophyte within cells of *Elaeagnus* inoculated with *Frankia* sp. EuI1. Note lack of endophytic vesicles. *c*, Actinomycete within cells of *Alnus* inoculated with *Frankia* sp. AvcI1. Vesicles (small arrows) are shown embedded in coagulated host cell cytoplasm.

nodules initiated by the *Alnus* isolate contained the vesicular endophyte typical of the *Alnus* symbiosis (Fig. 2*c*). These results suggest that the ineffective nature of the *Elaeagnus* isolate is peculiar to that organism and is not a result of the isolation procedure.

The technique described here is a relatively simple procedure for the isolation of actinomycete endophytes. The use of sucrose-density fractionation has also been applied to rhizobial–legume symbioses[9,10] and we are attempting isolations from other actinomycete-nodulated plants using this technique. Density sedimentation has proved superior to a previously used fractionation technique[11] for endophyte isolation.

The actinomycetes that we have isolated and cultured *in vitro* show a striking morphological similarity to the *Elaeagnus* and *Alnus* endophytes found in nodules of field-collected plants and each is able to reinfect the corresponding seedlings grown in water culture.

The fact that the *Elaeagnus* isolate does not form vesicles and does not reduce dinitrogen is particularly interesting in view of the postulated role of the vesicles in nitrogenase activity. Vesicles have been implicated as the site of nitrogen fixation[12] and previous research has shown that ineffective, non-vesiculate strains may exist[13]. The fact that this infective actinomycete fails to form vesicles and is incapable of fixing atmospheric nitrogen strengthens the hypothesis that the vesicle is the site of the nitrogenase enzyme.

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Dual origin of recent Dutch elm disease outbreaks in Europe

C. M. Brasier

Forest Research Station, Alice Holt Lodge, Wrecclesham, Farnham, Surrey, UK

Dutch elm disease was first recorded in 1918 in Western Europe and quickly spread to the US. The origin of the causal fungus, *Ceratocystis ulmi*, is unknown. A serious new epidemic began in the UK around 1970 and led to the discovery that there were two strains of the fungus: a highly pathogenic 'aggressive strain' responsible for the epidemic and thought to have been imported from North America[1,2], and a less pathogenic 'non-aggressive strain' believed to be endemic to the UK and adjacent parts of Europe since the 1920s (refs 1, 2). Recent disease outbreaks associated with the appearance of the aggressive strain in Holland[3] (1972), France[4,5] and Germany[6,7] (1973) were assumed to be secondary foci of the UK epidemic. A similar outbreak in Italy[7] (1973), however, seemed to be of longer standing (L. Mittemberger and O. Rackham, personal communications) and this, together with the discovery of the aggressive strain in Iran in 1975 (ref. 8), suggested that there was more than one source of the aggressive strain. I show here that there are two distinct races of the aggressive strain and that they have probably entered Europe independently.

When the colony morphologies of aggressive isolates were examined, those from the US, Canada, Holland, France and Germany were found to resemble closely UK isolates, being regular and striate, whereas those from Italy were uneven and less striate. Moreover, isolates obtained from Iran in 1975 were of the same morphological type as the Italian isolates, and a survey in 1977 confirmed the widespread occurrence of the Italian type in Iran[8].

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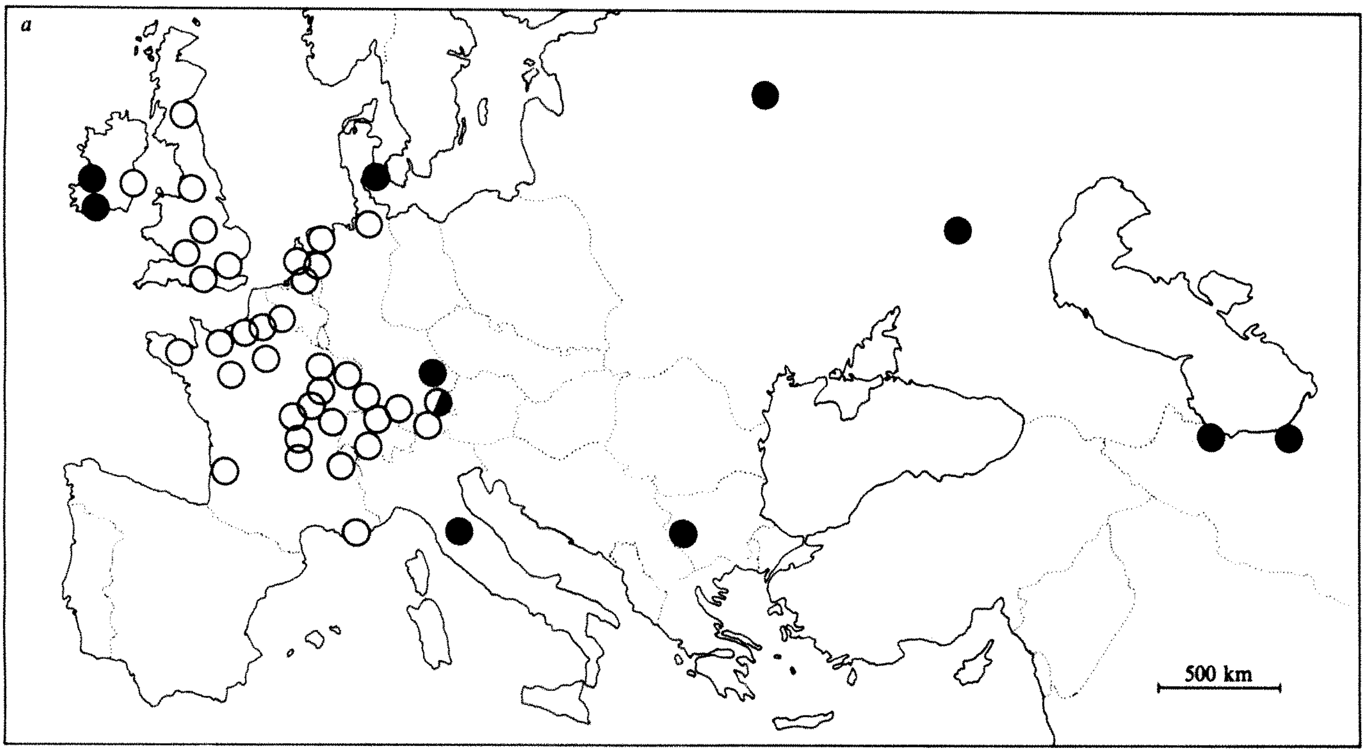

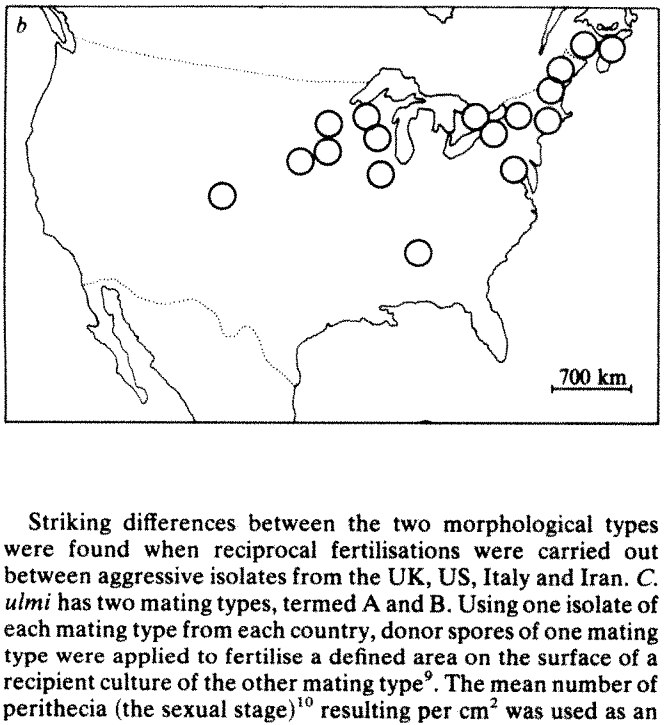

Fig. 1 Known distribution of the NAN (○) and EAN (●) races of the aggressive strain of *C. ulmi* in Europe and Asia (*a*) and North America (*b*) at the end of 1978.

Striking differences between the two morphological types were found when reciprocal fertilisations were carried out between aggressive isolates from the UK, US, Italy and Iran. *C. ulmi* has two mating types, termed A and B. Using one isolate of each mating type from each country, donor spores of one mating type were applied to fertilise a defined area on the surface of a recipient culture of the other mating type[9]. The mean number of perithecia (the sexual stage)[10] resulting per cm^2 was used as an index of the relative fertility of a given combination. The UK and US isolates were highly fertile with all donor isolates, whereas Italian and Iranian isolates showed low fertility with the UK and US isolates and high fertility with each other, the latter combinations being on average 18 times more fertile (Table 1). This indicated that the two morphological types were separate genetic entities, and they were provisionally termed the North American (NAN) and Eurasian (EAN) races of the aggressive strain.

Further samples of the aggressive strain were obtained from sites throughout North America and Europe. They included isolates from new outbreaks associated with the appearance of the aggressive strain in Ireland[11] (1977) and Denmark (1978) and isolates from the USSR and Bulgaria. Of 201 isolates examined morphologically, 127 were classified as NAN and 74 as EAN (Table 2).

To investigate the relationship between morphological type and fertility, a sample of 54 EAN and 55 NAN isolates from various countries were mated against NAN test isolates from the UK and US, and EAN test isolates from Italy and Iran. The NAN isolates again showed very low fertility against the EAN testers. All other combinations were uniformly highly fertile. The EAN × EAN (tester) combinations were on average 14 times more fertile than the NAN × EAN (tester) combinations (Table 2). Evidently, a reproductive barrier is operated unidirectionally by the EAN against the NAN isolates, a classic form of genetic isolation. The clear correlation between morphological type and relative fertility confirmed the NAN and EAN as separate genetic entities.

The NAN is the morphological type originally described in 1973 (ref. 1). The EAN differs from the NAN in having a less distinctive striate–petalloid pattern, the colonies often being lobed and uneven. In growth rate tests at 20 °C EAN isolates grow marginally more slowly than NAN isolates. Fresh EAN isolates often sector to a 'uniform powdery' (*up*) mutant, thought to be cytoplasmic in origin and the cause of their unevenness and slower growth.

That the NAN and EAN should be considered separate races within the aggressive strain rather than separate strains of *C. ulmi* is demonstrated by their similarities. In a preliminary pathogenicity test both types were highly pathogenic to *Ulmus procera*. In each, the B-mating type predominates and the rarer A-type characteristically produces protoperithecia on malt agar[8–10]. Both exhibit the unusual phenomenon of pseudoselfing[10]; both show extensive genetic isolation from the non-aggressive strain (refs 9 and 12 and C.M.B., unpublished); both have an optimum temperature for growth of ~20–22 °C compared with ~30 °C for the non-aggressive strain (J. Lea and C.M.B., unpublished).

As Fig. 1 shows, US, Canadian, UK, French, Dutch, Austrian and Swiss isolates are exclusively of the NAN race whereas Italian, Danish, Bulgarian, USSR and Iranian isolates are

Table 1 Frequency of perithecia (mean per cm^2) in inter-fertility tests of aggressive isolates of *C. ulmi* from the UK, US, Italy and Iran

		B-type donor isolates				*t*-tests for differences between means of UK and US against Italy and Iran
		UK	US	Italy	Iran	
A-type recipent isolates	UK	451	387	381	423	NS
	US	189	186	228	173	NS
	Italy	*3*	*0*	56	41	†
	Iran	*75*	*29*	246	305	*
		A-type donor isolates				
		UK	US	Italy	Iran	
B-type recipeient isolates	UK	198	281	239	284	NS
	US	43	24	30	32	NS
	Italy	*115*	*55*	357	368	*
	Iran	*32*	*5*	309	251	†

The experiment was carried out on elm sapwood agar. Each figure is the mean of four replicates. Student *t*-tests were carried out on replicate data transformed to square roots; significance levels: NS, not significant; * $P = <0.01$; † $P = <0.001$.

exclusively EAN. With the exception of Ireland, therefore, NAN shows a distinctly westerly and EAN a distinctly easterly distribution, both races occurring at Rosenheim in south-east Germany (5 EAN and 5 NAN in a sample of 10 isolates in August 1978).

On this evidence I suggest that the recent outbreaks of Dutch elm disease in Western Europe are of dual origin: through the importation of the NAN race of the aggressive strain from North America into the UK in the late 1960s (ref. 2), and into France, Holland and Germany, probably via Britain, in the early 1970s; and through migration of the EAN race from Italy, from central Europe or from farther east.

The Irish outbreak of 1977 is particularly interesting. The NAN isolates came from the Dublin area, whereas the EAN isolates all came from the Limerick–Cork area in the south-west. The Dublin and Limerick outbreaks were thought by Walsh and Mangan[11] to have originated independently, and the geographical distribution of the two races seems to confirm this. It would be intriguing to know the origins of these outbreaks. The Danish outbreak of 1978 is so far confined to Odense on the island of Funen, and because it is caused by the EAN race is probably due to importation of diseased material from the south or east. These events indicate that had the aggressive strain not reached the UK in the form of the NAN race from North America, it would probably have eventually arrived in the form of the EAN race from south or central Europe.

Table 2 Separation of aggressive isolates of *C. ulmi* into the NAN and EAN morphological types, and results of fertility tests against a recipent EAN isolate

	Morphological type			Fertility tests against EAN isolate* Mean perithecia per cm^2	
Country	No. of isolates examined	No. of NAN	No. of EAN	NAN isolates (no. of isolates tested)	EAN isolates
Austria	2	2	—	3.5(2)	—
Bulgaria	5	—	5	—	95.0(5)
Canada	7	7	—	3.5(2)	—
Denmark	3	—	3	—	70.4(3)
France	45	45	—	2.7(15)	—
Germany	22	15	7	2.1(6)	41.2(7)
Holland	13	13	—	5.5(8)	—
Ireland	27	7	20	5.9(7)	49.3(20)
Iran	32	—	32	—	35.6(13)
Italy	4	—	4	—	43.7(3)
USSR	3	—	3	—	58.7(3)
Switzerland	2	2	—	5.0(3)	—
US	36	36	—	2.1(6)	—
UK	(>4,000)	(>4,000)	—	4.0(8)	—
			Mean	3.6(55)	50.6(54)

* Isolates were from twig samples from individual diseased trees with the exception of two from the USSR which were bark isolates. Over 4,000 twig and bark isolates have been examined in the UK since 1972.

† The recipient EAN isolate used as a standard was Iranian isolate AST 20 ('A' mating type). All isolates tested against it were 'B' mating type. The data are from five separate experiments carried out on elm sapwood agar.

Further surveys of the fungus are now required in Europe and Asia, in particular Siberia, the Himalayas and China. These are the main sources of resistant elm material and somewhere in this region may be the centre of origin of *C. ulmi*. The full extent of variation in the fungus needs to be determined as soon as possible for the purposes of breeding resistant elms. Promising elm material should now be screened with both races of the aggressive strain.

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Energy dependence and reversibility of membrane alterations induced by polyene macrolide antibiotics in *Chlorella vulgaris*

Barbara Malewicz & Edward Borowski

Department of Pharmaceutical Technology and Biochemistry, Technical University, Gdańsk and Institute of Oceanology, Polish Academy of Science, Poland

The requirement of metabolic energy for the interaction of polyene macrolide antibiotics with eukaryotic organisms remains a controversial subject (for review see ref. 1). It has been claimed that the lethal binding of these antibiotics to the sterol target component of the hydrophobic core of the membrane, in accordance with the model of de Kruijff and Demel[2], is an energy-dependent process. When energy production is reduced by removal of all metabolisable substrates or by adding metabolic inhibitors, polyene binding and antifungal effects are also reduced. Metabolic energy may be required to maintain binding site accessibility or to move antibiotic molecules to the active site. The interaction is also restricted at low temperatures, possibly because of the reduced thermal mobilities of the groups concerned with antibiotic uptake. However, it should be emphasised that the interaction of polyene macrolides with artificial lipid membranes is a purely physicochemical process, although the type of permeability pathways induced are similar to those observed in natural membranes[1]. Using *Chlorella vulgaris* as a model organism, we demonstrate here that the interaction of polyene macrolides with sensitive cells and the induction of lethal membrane permeability changes are energy-dependent processes or purely physicochemical phenomena, depending on the structure of the antibiotic used.

As *C. vulgaris* is an autotrophic aerobic organism, energy production can be controlled naturally by changing the availability of light and oxygen, thus eliminating the need to use

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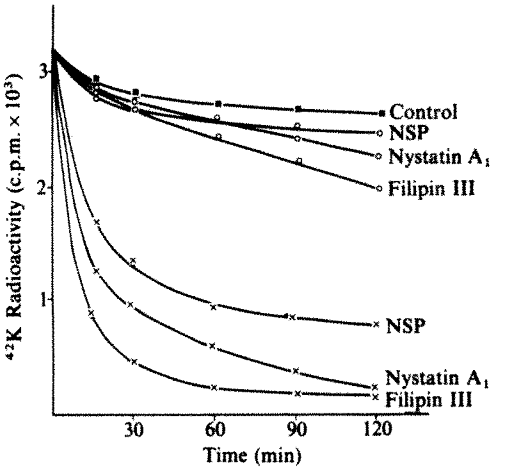

Fig. 1 The influence of temperature on the leakage of potassium from *C. vulgaris* on treatment with filipin III (30 μg ml^{-1}), nystatin A$_1$ (10 μg ml^{-1}) and *N*-succinylperimysin (NSP) (25 μg ml^{-1}). ^{42}K-labelled *C. vulgaris* cells (3 mg ml^{-1}) were suspended in Tris-HCl buffer, *p*H 7.2, at 0 °C (○) or 30 °C (×), and 0.1-ml samples of antibiotic solution in dimethyl sulphoxide (DMSO) added. The suspensions were aerated by shaking and illuminated. Aliquots were taken, cells filtered and washed with ice water and their radioactivity recorded.

chemical inhibitors which are not usually very specific. In anaerobic conditions and in the absence of light, ATP production in *Chlorella* spp. is completely inhibited[3]. The presence of ergosterol in the cytoplasmic membrane[4] makes this organism sensitive to the action of polyene macrolides[5,6].

In this study we have used compounds which represent possible different types of interaction with *C. vulgaris*, based on the reversibility of the lethal action of polyene macrolides on this organism. Following our observation on the reversibility of fungicidal action of a polyene macrolide[7], we have demonstrated that the ability of *C. vulgaris* cells to repair antibiotic-induced damage[6] depends on the structure of these antibiotics. Small macrolide ring polyenes (for example, filipin III) act irreversibly, whereas the action of those with a large macrolide ring but with low unsaturation in the chromophore (for example, nystatin A$_1$) can be reversed after brief contact with cells but not after prolonged exposure. Compounds with a large macrolide ring and a heptaenic chromophore (for example, *N*-succinylperimycin) induce repairable membrane alterations, and their lethal action can be fully reversed after removal of the antibiotic from the medium, regardless of the time of exposure.

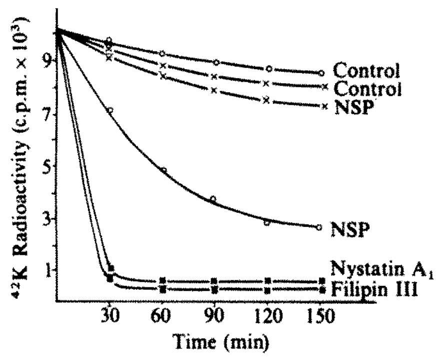

Fig. 2 The influence of the metabolic inhibitor, CCCP, on the potassium leakage from *C. vulgaris* treated with filipin III (50 μg ml^{-1}), nystatin A$_1$ (20 μg ml^{-1}) and *N*-succinylperimycin (NSP) (25 μg ml^{-1}). ^{42}K-labelled *C. vulgaris* cells (3 mg ml^{-1}) were suspended in Tris-HCl buffer *p*H 7.2, at 30 °C and preincubated with (×) and without (○) 5×10^{-5} M CCCP for 30 min with shaking and illumination, followed by the addition of antibiotics in 0.1 ml DMSO. Aliquots were taken, cells filtered and washed with ice water and their radioactivity recorded.

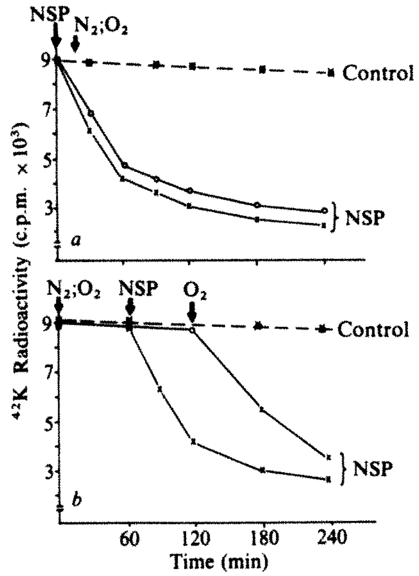

Fig. 3 The influence of light and oxygen on potassium leakage from *C. vulgaris* on treatment with *N*-succinylperimycin (NSP) (25 μg ml^{-1}). ^{42}K-labelled *C. vulgaris* cells (3 mg ml^{-1}) were suspended in Tris-HCl buffer, *p*H 7.2, at 30 °C. *a*, The suspension was aerated by shaking and illuminated, then a 0.1-ml solution of NSP in DMSO added. After 15 min, nitrogen was passed through the suspension in the dark (○) or the culture was kept in the light and aerated (×). Aliquots were taken, the cells filtered, washed with ice water and dried, and their radioactivity recorded. *b*, The suspension was kept in nitrogen in the dark, with shaking, or aerated under illumination. After 1 h NSP, 25 μg ml^{-1}, in 0.1 ml DMSO was added to both suspensions. After another hour, air was introduced to the anaerobic suspension and the suspension was shaken and illuminated. Aliquots were taken from both suspensions, the cells filtered, washed with ice water and dried, and their radioactivity recorded. ○, N$_2$; ×, O$_2$.

The interaction between the compounds examined and *C. vulgaris* was followed by measuring the leakage of potassium ions. The discharge of the gradient of this marker ion may be regarded as one of the first indications of membrane damage[8]. Filipin III[9], nystatin A$_1$ (ref. 10) and *N*-succinylperimycin[11,12] induce potassium leakage only at elevated temperatures (Fig. 1). In these conditions the use of the metabolic inhibitor carbonyl cyanide *m*-cholorophenylhydrazone (CCCP) does not prevent the interaction of filipin III and nystatin A$_1$ with *C. vulgaris*, but strongly inhibits the activity of *N*-succinylperimycin (Fig. 2). The leakage of potassium from cells treated with filipin III and nystatin A$_1$, in the presence of CCCP, indicates that the interaction of these antibiotics with the plasma membrane of *C. vulgaris* is a physicochemical phenomenon, not an energy-dependent process. The energy dependence of the interaction of *N*-succinylperimycin with the membrane was further confirmed by changing the availability of light and oxygen (Fig. 3). Whether the cells were treated with *N*-succinylperimycin in aerobic conditions and after 20 min nitrogen passed through the suspension in the dark, or whether the culture was aerated in the light, the potassium efflux was practically identical (Fig. 3*a*). This indicates that once *N*-succinylperimycin has reacted it induces a permeability pathway for potassium, effective in the presence or absence of metabolic energy. On the other hand, when the suspension was well washed with nitrogen and kept in the dark before the addition of

N-succinylperimycin, no potassium leakage occurred even after a 1-h exposure to the antibiotic. The subsequent introduction of oxygen and illumination caused rapid discharge of the potassium gradient, similar to that seen when the antibiotic was added in aerobic conditions (Fig. 3*b*). This experiment clearly shows the requirement of metabolic energy for the induction by *N*-succinylperimycin of a potassium permeability pathway in *C. vulgaris*.

We conclude that the polyenes examined which induce irreversible membrane permeability changes in *C. vulgaris* (filipin III and nystatin A_1) interact with the membrane in a physicochemical manner. On the other hand, the compound whose lethal action can be reversed and the induced membrane damage repaired (*N*-succinylperimycin) requires metabolic energy for its interaction with the plasma membrane.

Our findings indicate that the formation of various types of membrane structures can be induced by polyene macrolides, depending on the structure of the antibiotic. Although very little is known about the structures created in biological membranes by polyene macrolides, hypothetical molecular models have suggested that bulky polyene–sterol complexes, half pores, or conductance channels can be formed on interaction of various polyene macrolides with sterol-containing lipid bilayers[2]. It is reasonable to assume that only the formation of a highly specific membrane structure, namely, the ion-selective conductance channel, requires metabolic energy. The formation of ion-specific channels by *N*-succinylperimycin is indicated by the complete reversal of its killing action in a medium containing an isotonic concentration of potassium salts, added to prevent death through lack of potassium[6,7].

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Ionophore A23187 disrupts membrane structure by modifying protein–lipid interactions

Richard D. Klausner, Mark C. Fishman & Morris J. Karnovsky

Departments of Medicine and Pathology, Harvard Medical School,
Boston, Massachusetts 02115

The physiological function of membrane-bound protein clearly depends on the nature of its neighbouring lipid[1]. However, the question of how critical the protein is to the structure and function of membrane lipid has received much less attention. There is some evidence that lipid surrounding membrane protein, 'boundary lipid', may be more ordered than continuum lipid[2]. Protein can also alter the enthalpy and temperature of pure lipid-phase transitions[3]. However, controversy surrounds the question of whether membrane proteins determine long-range aspects of lipid structure or merely perturb their local environments[4,5]. Here, we demonstrate that a lipophilic substance, the calcium ionophore, A23187 (Lilly), dramatically disorders the lipid structure of the membrane and that this phenomenon depends on the presence of proteins.

We used the polyene fluorescent probe, 1,6-diphenyl-1-3-5-hexatriene (DPH), to measure membrane lipid order[6]. Steady-state fluorescence polarisation was measured with an SLM model 4800 fluorescent phase modulation lifetime machine[7]. All measurements were corrected for scatter. Lifetime measurements were made with the same machine. Fluorescence spectra were obtained with a Perkin-Elmer model MPF-2a spectrophotometer. The probe was incorporated into cells (fresh splenic mouse lymphocytes, BHK or 3T3 cells grown in culture) and multilamellar lipid vesicles as previously described[8]. Vesicles were prepared from lipid extracts of cell membranes[9] or with purified phospholipids (Sigma). Purified (Na^+ + K^+)ATPase (Sigma) was reconstituted into lipid vesicles by solubilising both lipid and protein at a 10:1 (w/w) ratio in 10 mM Tris, 120 mM NaCl and 5% Na deoxycholate (DOC). The DOC was removed by dialysis for 72 h. Spectral analysis revealed that about 25% of the original protein could be incorporated into the resulting vesicles. Freeze-fracture electron microscopy of the reconstituted vesicles showed many intramembranous particles.

Figure 1 shows the dramatic DPH polarisation changes in lymphocytes as a function of the concentration of A23187. They suggest marked alterations in lipid packing due to the ionophore. The *P* value change occurred within 30 s and was not affected by the addition of EGTA or alteration of the concentrations of Ca^{2+} or Mg^{2+}. It was not inhibited by 10 $\mu g\ ml^{-1}$ of cytochalasin B, 10^{-6} M colchicine or 10^{-3} M NaN_3. The effect persisted despite washing and resuspending the treated cells, and was not abolished by heating the cells to 65°C for 15 min. This dose-dependent lowering of the *P* value was seen with all cell types tested. However, these polarisation changes were not found in pure lipid systems (even at 10^{-4} M A23187). Thus, as shown in Table 1, A23187 did not affect the *P* value of DPH-labelled liposomes. Reconstituted vesicles, on the other hand, containing protein and lipid, exhibited a diminution in DPH polarisation comparable to that of whole cell membranes.

Spectral analysis showed that there was no alteration in DPH excitation or emission spectra produced by the ionophore. Fluorescence lifetimes of DPH in cell membranes were measured. These were unaltered in the presence of the ionophore, indicating that the steady-state polarisation changes reflect true alterations in the rotational correlation times of the probe.

It surprised us that this lipophilic drug required protein to exert its 'fluidising' effect on membrane lipids. It is possible that the drug binds to proteins, decreasing the overall probe polarisation by displacing 'rigid' boundary lipid. However, extraction of membrane proteins and hence of boundary lipid results in only a 20% dimunition in DPH polarisation (Table I). This is

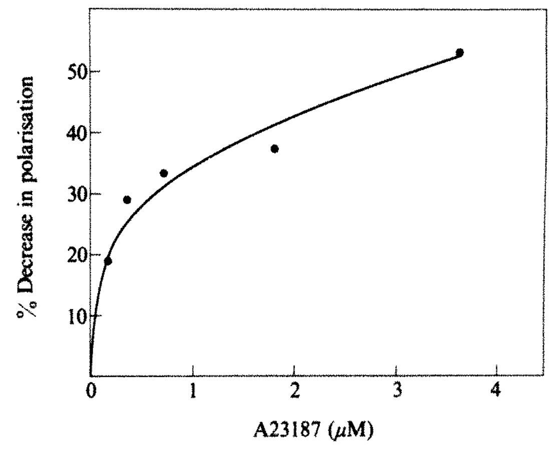

Fig. 1 Percentage decrease in polarisation of DPH as a function of the concentration of A23187 added to lymphocyte membranes. Baseline polarisation is 0.268 ± 0.012 (s.e.m.)

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Table 1 The effect of A23187 on DPH polarisation

Lipid	*P* Value	
	Control	+A23187
Phosphatidylcholine dimyristoyl		
4°C	0.440 ± 0.006	0.438 ± 0.005 (1 μM)
23°C	0.254 ± 0.014	0.250 ± 0.011 (1μM)
30°C	0.160 ± 0.008	0.160 ± 0.006 (1 μM)
Liposomes from:		
Lymphocytes	0.252 ± 0.012	0.250 ± 0.008 (1 μM)
BHK cells	0.268 ± 0.010	0.276 ± 0.006 (1 μM)
Liposomes reconstituted with ($Na^+ + K^+$)ATPase		
Lymphocytes	0.304 ± 0.018	0.220 ± 0.028 (0.72 μM) 0.178 ± 0.040 (1.44 μM)
BHK cells	0.280 ± 0.022	0.245 ± 0.020 (0.72 μM) 0.220 ± 0.019 (1.44 μM)

Polarisation of DPH in pure phosphatidyl choline vesicles above, at and below the phase transition is not altered by the addition of A23187. Liposomes made from lipid extracts of lymphocytes or BHK cells and tested at 23°C also seem to be unperturbed by the ionophore. Vesicles reconstituted with ($Na^+ + K^+$)ATPase show dose-dependent alterations in DPH polarisation with A23187. Values given as mean and s.e.m.

much smaller than the change induced by A23187. Thus, we consider this explanation unlikely. It is more likely that the ionophore can exert its disordering influence only when the lipids are in specific configurations. Thus, perhaps pure lipids cannot assume such structure, but the insertion of proteins reorganises the lipids into appropriate configurations. Analogies exist: for example, chlorpromazine alters lipid structure, but only in the presence of appropriate cholesterol content[10].

The nature of this special lipid configuration is unclear. However, it is unlikely to be simply the gel or liquid crystalline phases, because no effect is seen in pure phosphatidylcholine dimyristoyl vesicles either above or below the endothermic phase transition. Where does the ionophore act? One possibility is, at the lipid protein boundary. A23187 binds to both lipids and proteins[11] and may well have particular affinity for the lipid–protein interface. At the lipid–protein boundary it would not act to displace boundary lipid but to alter the nature of the lipid ordering effects exerted by the membrane protein. The magnitude of our results suggests that the effect of protein on the lipid, at least by means of a perturbing molecule, can be large.

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An incipient 3_{10} helix in Piv-Pro-Pro-Ala-NHMe as a model for peptide folding

Y. V. Venkatachalapathi & P. Balaram

Molecular Biophysics Unit, Indian Institute of Science,
Bangalore 560 012, India

The molecular mechanism of helix nucleation in peptides and proteins is not yet understood and the question of whether sharp turns in the polypeptide backbone serve as nuclei for protein folding has evoked controversy[1,2]. A recent study of the conformation of a tetrapeptide containing the stereochemically constrained residue α-aminoisobutyric acid, both in solution and the solid state, yielded a structure consisting of two consecutive β-turns, leading to an incipient 3_{10} helical conformation[3,4]. This led us to speculate that specific tri- and tetrapeptide sequences may indeed provide a helical twist to the amino-terminal segment of helical regions in proteins and provide a nucleation site for further propagation. The transformation from a 3_{10} helical structure to an α-helix should be facile and requires only small changes in the φ and ψ conformational angles and a rearrangement of the hydrogen bonding pattern[5]. If such a mechanism is involved then it should be possible to isolate an incipient 3_{10} helical conformation in a tripeptide amide or tetrapeptide sequence, based purely on the driving force derived from short-range interactions. We have synthesised and studied the model peptide pivaloyl-Pro-Pro-Ala-NHMe (compound I) and provide here spectroscopic evidence for a 3_{10} helical conformation in compound I.

The choice of the pivaloyl blocking group at the amino-terminal was dictated by the necessity of maintaining the first amide bond in a *trans* configuration. While X-Pro bonds can exist in both *cis* and *trans* configurations in solution[6], the bulky tertiary butyl group locks the piv–Pro bond into the *trans* structure[7]. ^{13}C NMR studies, of a number of pivaloyl-Pro containing peptides, show no evidence for the presence of the *cis* isomer (unpublished observations). The Pro-Pro-Ala sequence was chosen because of the strong tendency of Pro-X sequences to form β-turns[8], a consequence of the restriction of the φ angle in the pyrrolidine ring to ~ −60°. The carboxyl terminal was converted to the methylamide to provide the NH group necessary for stabilising the second β-turn by formation of an intramolecular 4 ← 1 hydrogen bond.

Figure 1*a* and *b* shows the 270 MHz ^1H and 67.89 MHz ^{13}C NMR spectra of I in $CDCl_3$. It is clear from both spectra that of the four possible isomers about the pivaloyl-Pro and Pro-Pro bonds (*trans–trans, trans–cis, cis–trans* and *cis–cis*) only one is present in solution. The chemical shifts of the C^β and C^γ resonances of both Pro residues (Table 1) fall within the range expected for *trans* X-Pro bonds[9], suggesting that both the pivaloyl-Pro and Pro-Pro bonds are exclusively *trans*. The effect of changing temperature and solvent on the chemical shifts of the Ala and methylamide NH groups, are summarised in Table 1. Both amide hydrogens show a very small temperature coefficient in $(CD_3)_2SO$ and almost no change in chemical shift on going from a poor hydrogen accepting solvent, such as $CDCl_3$, to a good acceptor, such as $(CD_3)_2SO$.

These results provide clear evidence that both NH groups in the molecule are shielded from the solvent[9]. The IR spectrum of I in dilute $CHCl_3$ solution shows the presence of only one N–H stretching band at 3,340 cm^{-1}, demonstrating that the NH groups are involved in the formation of intramolecular hydrogen bonds. The lack of a band at ~3,430 cm^{-1} suggests that conformations with free NH groups are absent. Figure 2 shows a possible conformation of compound I with the Ala and methylamide NH groups involved in 4 ← 1 intramolecular hydrogen bonds. While the available data only provide evidence for the participation of NH groups in hydrogen bonds, we propose the consecutive β-turn structure taking into account the known propensity of Pro residues to initiate β-turns[8]. Indeed the absence of a free NH stretching band at ~3,450 cm^{-1} argues for

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Table 1 NMR parameters for pivaloyl-Pro-Pro-Ala-NHMe

	NH chemical shifts (δ)		$\Delta\delta$ (p.p.m.) per °C*		$J_{C^\alpha H\text{-}NH}$ (Hz)	^{13}C chemical shifts	
Solvent	Ala	$NHCH_3$	Ala	$NHCH_3$	Ala	C^βPro	C^γPro
$CDCl_3$	7.37	7.01	2.5×10^{-3}	2.9×10^{-3}	9.2	27.16 29.64	24.55 26.69
$(CD_3)_2SO$	7.39	7.07	1.5×10^{-3}	1.6×10^{-3}	8.9	26.52 27.07	23.98 26.05

Spectra were recorded on a Bruker WH-270 FT-NMR spectrometer at the Bangalore NMR facility. ^1H and ^{13}C chemical shifts are expressed as δ (p.p.m.) downfield from the corresponding resonances of internal tetramethylsilane.

* Temperature coefficients of NH resonances.

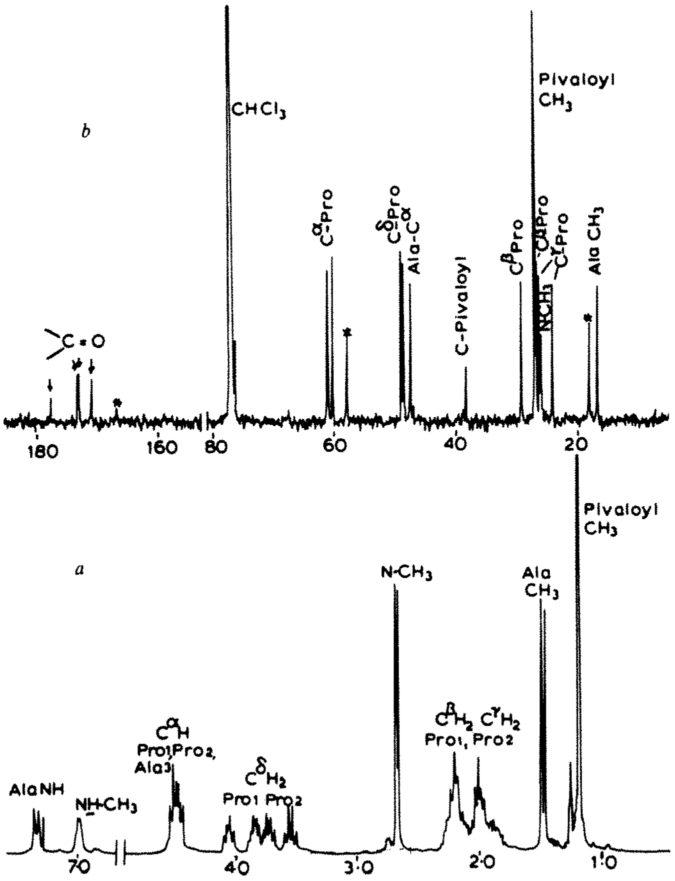

Fig. 1 *a*, 270 MHz ^1H NMR spectrum of compound I in $CDCl_3$. *b*, 67.89 MHz ^{13}C NMR spectrum of I in $CDCl_3$. Starred peaks are due to impurities in the solvent.

an almost exclusive preference for the highly folded incipient 3_{10} helical structure shown in Fig. 2. The observed $J_{NH\text{-}C^\alpha H}$ values of 9.2 Hz and 8.9 Hz in $CDCl_3$ and $(CD_3)_2SO$ for the Ala NH are compatible with a ϕ value of $\sim -80°$ for the Ala residue[10]. The presence of two Pro residues with $\phi_{Pro} \sim -60°$ and the constraints of two intramolecular $4 \leftarrow 1$ hydrogen bonds suggested that ψ_{Pro} values[11] are likely to be restricted to the range $-20°$ to $-40°$. These values are very close to those expected theoretically in a 3_{10} helix ($\phi \sim -60°$, $\psi \sim -30°$).

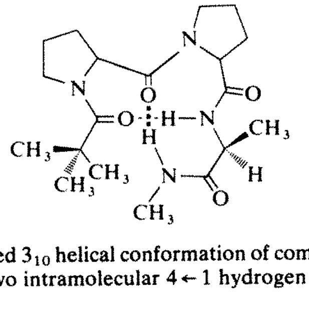

Fig. 2 Proposed 3_{10} helical conformation of compound I showing the two intramolecular $4 \leftarrow 1$ hydrogen bonds.

The importance of the methylamide NH group in stabilising this folded structure is emphasised by the fact that in the related compound pivaloyl-Pro-Pro-Ala-OCH_3, the presence of three conformers in the ratio 37 : 28 : 35 can be detected by 270 MHz ^1H NMR in $CDCl_3$. Further, the Ala $J_{NH\text{-}C^\alpha H}$ values of 7.3, 7.7 and 7.0 Hz in the three species reflect conformational averaging over the Ala N–C^α bond and may be contrasted with the J value obtained for I.

The above results provide direct evidence of a 3_{10} helical structure in a tetrapeptide containing some amino acids that occur frequently in proteins. In view of the frequent observation of 3_{10} helical segments at the ends of α-helical regions in protein crystal structures[12], it seems that consecutive β-turn structures have a definite role in giving the polypeptide chain a helical twist. In particular, peptide sequences containing a cluster of residues with a strong tendency to occur in β-turns, like Pro, Gly, Asn, Asp, Ser and Cys[13], may have an important role in forming 3_{10} helical segments. Experimental tests of this hypothesis are under way.

We thank Ch. Pulla Rao for recording the IR spectrum and the University Grants Commission, India, for financial support.

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Corrigendum

In the letter 'Parity nonconservation and stellar collapse' by A. Vilenkin, *Nature* **280,** 569–570 it was argued that the direction dependence of the neutrino mean free time in a magnetic field **B** gives rise to a neutrino energy flux parallel to **B**. However, it can be shown from the Boltzmann equation that scattering reactions ($\mathbf{p}r, \mathbf{q} \rightarrow p'r', q'$) and ($\mathbf{p}'r', \mathbf{q}' \rightarrow \mathbf{p}r, \mathbf{q}$) where **p** and **q** are electron and neutrino momenta and *r* is electron spin, balance in such a way that the net result is zero; that is, there is no neutrino energy flux in a magnetic field. The author thanks Ch. G. Van Weert and L. van den Horn for a discussion which helped to reveal the error.

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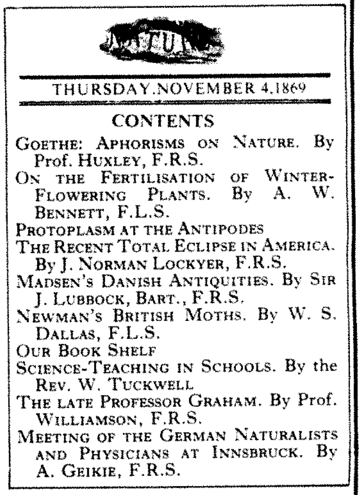

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reviews

Science policy in the developing world

Ziauddin Sardar

Science, Technology and Development: Essays in Honour of A. Rahman. Edited by K.D. Sharma and M.A. Qureshi. Pp. 478. (Sterling Publishers: New Delhi, 1978.) Rs 120.

THIS anthology of papers on the themes of science policy, science and society, technology assessment, technology and underdevelopment, research management and science in India is a fitting tribute to Professor A. Rahman, "whose pioneering work", in the words of J. J. Soloman, "in science policy studies related to the problems of development has been outstanding". The idea of honouring a Third World scientist who has made a varied and sustained contribution to knowledge is indeed appropriate and thee must certainly be others who would have liked to be associated with it.

Science, Technology and Development opens with a brief biographical sketch of A. Rahman. Rahman was educated at the Aligorh Muslim University and in England where he studied biochemistry under the Noble Laureate H.A. Cripps and came in contact with such scholars of the left as J.D. Bernal, Joseph Needham, J.B.S. Haldane and H. Levy. Their influence has obviously left its mark on his thinking and is responsible for his switch from biochemistry to the history and social philosophy of science. Rahman's distinguished career in the Council of Scientific and Industrial Research (CSIR) began at Regional Research Laboratory in Hyderabad immediately after his return from England. Since then he has served in many CSIR laboratories, moving, in 1963, to CSIR Headquarters in New Delhi to organise an information centre and to develop a systems approach to planning and resource allocation for the various laboratories of CSIR. Currently, he is Chief of Planning at CSIR and leads the Centre for the Study of Science, Technology and Development.

There are 26 papers in this presentation volume, inevitably of uneven quality. Some of the essays (for example, J. J. Soloman on "Science Policy and Social Objectives", E. B. Skolnikoff on "The Governability of Complexity", A. H. Rubenstein, T. W. Schlie and A. D. Jedlicka on "Some Current Field of Research on "Appropriate Technologies for the Less Developed Countries" and S. R. Mikulinsky on "The Present State and Problems of the History of Natural Science") seem to be positively dated, some others (for example, Y. de Hemptine on "Integrated versus Sectoral Policies for Science and Technology", Jozef Bognar on "Culture, Science and Economy" and D. de Solla Price on "India as a Small Highly Developed Scientific Nation") are in the nature of fragments only; but, on the whole, there is enough thought provoking material here for the volume to be valuable to both the students and researchers of the social relations of science and technology.

The volume is at its strongest when dealing with science and technological policy in India. In a commanding analysis, A. K. N. Reddkly argues for the generation of environmentally sound and appropriate technolgies for rural development. His strategy is based on two components: forging strong links between the educational, scientific and technological institutions and the needs of the rural poor and their traditional technologies, on the one hand, and drastically weakening the reliance of these institutions on elitist demands and on institutions in the developed world. A strategy after my own heart, and one that C. Subramaniam shows so convincingly is bound to be fruitful.

Subramaniam describes a rural strategy developed for Karimnager, "a backward district of Andhra Pradesh". The work in Karimnager began with an assessment of soil, geohydrological and forest resources, an assessment of human potential, identification of new irrigation wells and a determination of fertilizer doses, and proceed to the planning of feeder roads and housing colonies based on techniques that emphasise maximum use of local material and skills. A windmill was designed and built for lifting water from the only source of drinking water in the village, carried out with the help of the National Aeronautical Laboratory, "which till then used to interest itself in nothing less lowly than designing and testing aeronautical systems for jet aircrafts". Other projects developed for Karimnager included the collections of all waste material — human, animal and agricultural — to be processed in an inexpensive biogas plant to produce domestic fuel and fertiliser, the substitution of rubber-roller type of shellers for the more conventional energy: sheller in rice processing equipment, and improvement of various techniques from bee-keeping, making palm-candy to tanning and securing a better extraction of fibre from banana and sisal. Karimnager, K. Subramaniam argues forcefully, is a shining example of what can be achieved when scientists turn their attention to the needs of the rural poor.

India is saturated with Karimnagers and it is reasonable to assume that this success story will be repeated elsewhere. Indeed, as K. D. Sharma and M. A. Qureshi argue in their contribution, "A Framework for Alternative Technology", India needs to develop a mix of "traditional small and big technologies to achieve composite development so as to strike a balance between the internal socio-economic situation and external plolitical stability". Although "national prestige" and "national security" figure large in their thinking, Sharma and Qureshi are really arguing for an upgrading of traditional technologies and a little more scrutiny of high technology projects. There is little doubt that India cannot tolerate, as Ward Morehouse shows in his richly detailed study of "Scuttling the Coal Gasification Project at Hyderabad: The Perils of Public Patronage in Bridging the Development Gap", a situation where its science is unwittingly pushed forward in large, prestigous undertakings. Scientists like Rahman, who turn their attention from the glare of prestige science and high technology to the plight of the rural poor and the real issues of development, deserve commendation from all who put people before glory. ☐

Ziauddin Sardar is an Information Consultant to the Hajj Research Centre, King Abdul Aziz University, Jeddah, Saudi Arabia.

Chemistry at the University of Edinburgh

The Playfair Collection and the Teaching of Chemistry at the University of Edinburgh, 1713-1858. By R. G. W. Anderson. Pp.175. (The Royal Scottish Museum: Edinburgh, 1978.). Paperback £4.50.

SHORTLY after Lyon Playfair's appointment in 1858 to the Edinburgh Chair of Chemistry he presented to what is now the Royal Scottish Museum 75 diverse pieces of scientific apparatus from the University Chemical Laboratory which he believed to be of historical significance. Sixty-four of these items, thought to be associated with Black, Hope and Gregory, are extant and comprise the Playfair Collection. It is an important collection, as few pre-nineteenth century chemical artefacts of known provenance survive.

Dr Anderson devotes about half of his book to dicussing the provenance of the Playfair Collection as a whole and to describing fully each item, the first detailed discussion of its kind to be published. To provide a context for this catalogue the first half of the book describes the teaching of chemistry at Edinburgh from 1713 to 1858; during most of this period the University of Edinburgh was pre-eminent in Britain in the teaching of Chemistry, thus making Anderson's book of interest to both students of early scientific apparatus and historians of science.

In the catalogue section, the entry for each item consists of physical description, illustration, brief history of the development of the class of apparatus and, for some items, evidence for associating it with a particular Professor. The illustrations, both photographs and diagrams, are excellent and the brief histories are scholarly and well-referenced. The very difficult task of substantiating the association of a particular item with a particular Professor is capably done but, as is probably inevitable, the evidence is sometimes less than convincing.

The first half of the book, the author states, is not intended to be a history of the teaching of chemistry at Edinburgh or of those who taught it. This section deals primarily with their laboratories, their acquisition of apparatus and, to a certain extent, what and how they taught. In his principal aims the author succeeds admirably while supplying sufficient biographical material to make a continuous and readable account; it is particularly valuable to have hitherto unpublished information on the first holder of the Chair, Crawford, and extensive new information on his successor, Plummer, both of whom, other than in Underwood's *Boerhaave's Men*, have received little scholarly attention in contrast to their successors, Cullen, Black, Hope and Gregory. Anderson's account is well-referenced, mainly to primary sources, and is very lucid. The locations of the various laboratories are identified with very extensive evidence. Perhaps of wider general interest are the types of apparatus acquired, the costs thereof and the sources of supply; here again the author is to be congratulated on the range and depth of his research. Less well done is the account of the teaching which, though adequate as to the method of teaching, gives little information on the content of the courses. There are a few minor errors: for example, Crawford was allotted money not "to put up his stalls" (p4) but his "stells", Old Scots for "stills"; again, Lyon Playfair was a candidate for the Edinburgh chair in 1844 (p55) as the Town Council Minutes relating to that appointment show.

One matter of opinion on which I must express disagreement with the author is his view that *Opera Chemica* is a student notebook of Plummer's lectures. I believe it to be a set of instructions for the pharmaceutical preparations carried out in Plummer's laboratory; it is difficult to believe from Plummer's two published chemical papers that his chemical lectures were so narrow as to consist merely of pharmaceutical processes. These minor criticisms do not detract from my view that this is a splendid book which will interest not only the specialist but also the general reader and which at £4.50 is remarkable value.

W. P. Doyle

W. P. Doyle is Senior Lecturer in Chemistry at the University of Edinburgh, UK.

Origins uncertain

The Origin of the Solar System. Edited by S. F. Dermott. Pp. 668. (Wiley: Chichester, UK, and New York, 1978.) £28.50.

WE know and understand less about the Earth's interior than the Sun's. We know less about the origin of the planets than we do about the formation of stars and even (one may feel) of the Universe. This will be apparent to anyone who reads *The Origin of the Solar System,* the proceedings of a NATO Advanced Study Institute held at the University of Newcastle in 1976, and capably edited by S. F. Dermott.

The reason for this state of affairs is clear enough: we have only one example of a planetary system with closely measured physical properties, whereas we can observe a large number of stellar systems (and it is difficult to falsify statistically predictions about the Universe). Nevertheless, it leaves one uneasy to see a field of science so dominated by speculation, with so little hard observational foundation. For that reason one cannot escape a sense of unreality when reading this volume, which could not have been readily remedied in 1976. The discovery since then of strong candidates for pre-planetary discs around a handful of nearby stars by the Steward Observatory's infrared observing group gives some grounds for hoping that we may be able eventually to test hypotheses against planetary systems observed now to be in the process of forming. Certainly we are learning much about the Sun's birth processes — a subject not given much prominence in the present book — from recent observations of currently forming stars.

Within the book one does find various contributions which are well written and which may serve as excellent introductory reviews of specialised topics. The article by Kirsten on the use of radioactive decay for chronology using samples from the Earth, the moon and meteorites starts with an admirable discussion of principles and their application, and contains exact descriptions of a variety of experiments. One does not need to agree with the minutae in his final cosmochronology to be impressed with the precision, and the well understood limits to that precision, of estimates such as 4.5 to 4.6 x 10^9 yr for the epoch of Solar System formation. A complementary paper by Larimer on meteorites in an essentially similar style is also thorough and readable. In a very different type of article Stevenson sets out clearly how global physical observations of the outer planets — their mean densities, gravitational moments and their recently measured internally generated luminosities — set constraints on their physical and chemical structures. One is then left with the problem of how the resulting abundance ratios of hydrogen to helium and to heavier elements came to deviate from their cosmic and especially their solar values. Neglecting such cosmogonic considerations we have models for the interior of Jupiter more reliable and more able to describe observable phenomena (principally its huge magnetosphere) than comparable models for the Earth.

It is on the theoretical side where credibility wears thin. Reeves acknowledges this in his introduction, where he outlines four types of theories for planetary formation, two of which were postulated in their original forms in the eighteenth century, but all of which have their contemporary protagonists.

These are the single nebula hypotheses of Kant (1755) and Laplace (1796), the accretion theory of Berkeland (1912), the exploding binary component model originally proposed by Gunn (1932) and the two-star tidal filament picture first proposed by Buffon (1745). Each has been fully articulated by modern physicists (Hoyle, for example, has contributed to all of them), who have introduced much inventive detail. The earlier chapters of this book, written by authorities such as Cameron, McCrea, Woolfson and Prentice provide well-written accounts of particular modern variants of these theories. Each is able to account, more or less, for the principal features of the Solar System: the co-planarity of planetary orbits, the contrary imbalances of mass and angular momentum between Sun and planets, the Titius-Bode orbit relationship, and planetary chemical compositions. Each such chapter is well-reasoned and persuasive, with the exception of an irritatingly dogmatic essay by Alfvén, ostensibly critical of myth in science, but guilty of more unsupported assumption that any of the theories criticised.

Considered as a snapshot of the way the subject looked in 1976, and as a wide-ranging introduction to various aspects of the subject for students with a physics background, the book has much to recommend it. It is also a valuable case-study of the Popperian dictum that a necessary criterion for judging a scientific theory is its experimental or observational falsifiability. Although much of the theoretical work presented yields predictions which are falsifiable in principle, the authors must be aware that they are safe in practice, at any rate for the next few years. Only time and improved instrumentation will reveal the extent of their self-indulgence.

John Beckman

J. E. Beckman is in the Department of Physics at Queen Mary College, University of London, UK.

Cancer for the layman

The Wayward Cell. Cancer: Its Origins, Nature, and Treatment. Second edition. By V. Richards. Pp. 407. (University of California Press: Berkeley, Los Angeles and London, 1978). £8.75.

The Wayward Cell is a most interesting and unique treatise on various aspects of oncological diseases, their origins, nature and clinical care. It aims to provide facts and figures about cancer which are easily understandable to the intelligent layman as well as those involved with paramedical disciplines. However, I believe that this book will also interest and stimulate clinical oncologists, both those who are established and those in training.

The idea for the book almost certainly was born from the frustrations which clinicians not infrequently experience both with respect to their own failure to cure some tumours and with the need to provide clear concise explanations of the protean manifestations of cancer often sought by their patients.

This volume is divided into four main sections dealing with the biology, clinical aspects and treatment of cancer and some of the social problems it causes. The biology part is concerned with aspects of cell growth and differentiation together with modern views of the importance of molecular biology in enabling us to understand how cells work.

The clinical section is perhaps the best part of the book with a fascinating and informative account of the history of cancer. The importance of exposure to environmental carcinogens in causing the numerous cancers of this day and age is dealt with logically and clearly together with a simple account of the ways in which such carcinogens might influence cell growth and function. It is perhaps a pity that this section contains no simple pathological classification of the various types of tumours to illustrate their diversity of structure and histogenesis.

The section on treatment will be of particular value to the non-medical person giving, albeit with perhaps undue emphasis on breast cancer, the background to the various therapeutic modalities in current use.

The final part of the book is called "Cancer — 1977" and is an attempt to present the current state of our knowledge, its deficiencies and the prospects for the future. This section is disappointing, being too detailed and scientific for the lay reader. Elements would have been better integrated with the other sections.

Overall, this is a good book, well written and excellently illustrated with clinical case reports to reveal the patient problems which tumours create. Very few books of this nature are available with such a wealth of information. It should interest the range of readers for which it is intended.

A. Munro Neville

A Munro Neville is at the Ludwig Institute for Cancer Research, Royal Marsden Hospital, Sutton, UK.

Selected immunological methods

Immunological Methods. Edited by I. Lefkovits and B. Pemis. Pp.488. (Academic, New York and London, 1979.) $36; £23.40.

Immunological Methods is a misleading title for this volume of techniques. A non-immunologist hoping to find recipes for standard immunolgical methods, such as immunodiffusion in agar and immunoelectrophoresis, will be disappointed. A better title would be: *Experimental Techniques used by Scientists at the Basle Institute for Immunology.* This title, although slightly long for the spine of the book, would justify the inclusion of the chapters on nanomole peptide mapping (B.A. Moss, chapter 3), the production of sendai virus (R. Tees, chapter 30), the running of an isotope laboratory (H.M. Pohlit *et al.*, chapter 33) and the excellent introduction to polyacrylamide slab gel electrophoresis (B. Takacs, chapter 4), all of which can hardly be described as immunological methods.

The editors have pursued a policy of allowing individual contributors freedom of style and content. This approach has resulted in a variation in depth of treatment afforded different topics and an emphasis on murine cellular immunology. For example, serological typing of human histocompatibility antigens is briefly treated in 10 pages (J.W. Stocker and D. Bernoco, chapter 14). An assay for specific alloantigen binding T cells activated in the murine mixed lymphocyte reaction is treated in detail and clarity in 17 pages (Elliott *et al.*, chapter 21).

Another problem resulting from the lack of editorial direction is the repetition of several techniques in different chapters, the most exteme example of which is the description of suspension cloning techniques for lymphoid cells in three chapters (M. Shulman, chapter 20; N.N. Iscove and M.H. Schreier, chapter 29; G. Köhler, chapter 32).

Among the better chapters in the volume is a delightfully written account of antibody binding characteristics (S. Fazekas de of Groth, chapter 1). Another bonus is the presence of techniques for making monoclonal antibodies (G. Köhler, chapter 31), although this latter chapter would have been more valuable if it had been updated to include PEG fusion techniques and extended to include different screening techniques.

This book can be commended to specialist immunologists, particularly cellular immunologists, but does not serve as an introduction to immunological techniques for the non-specialist.

Peter Goodfellow

Peter Goodfellow is a staff scientist at the Imperial Cancer Research Fund's Laboratories, London, UK.

Arthropod phylogeny

Arthropod Phylogeny: With Special Reference to Insects. By H.B. Boudreaux. Pp. 320. (Wiley: New York and Chichester, UK, 1979.) £15.

THE past fifteen years have seen the publication of major works on arthropod phylogeny by Sharov, Hennig, Anderson and, notably, by Manton. Professor Boudreaux has now added to them a book distinguished by two guiding principles. Firstly, he believes that the Arthropoda form a single monophyletic taxon and therefore sets himself firmly against the recent tendency to regard them as three or more lineages, each independently evolved from primitive lobopod ancestors. Secondly, as an advocate of Hennig's methods of phylogenetic reconstruction, he proceeds by defining monophyletic sister-groups strictly through their common specialisations and abandoning, without much hesitation, the paraphyletic and polyphyletic taxa of the past.

After treating the Onychophora as a phylum separate from but related to the Arthropoda, Boudreaux devotes almost a third of his book to the affinities of the arthropod classes and the remaining two-thirds to the mutual relationships of the insect orders. The great diversity of the phylum, the volume of factual material, and the closely reasoned interpretations defy a brief summary, but the book has the great merit of presenting explicitly the foundations (mainly more or less familiar morphological data from living forms) on which the inevitably somewhat speculative inferences are based. Essentially the author recognises two main branches in arthropod evolution: a Cheliceromorph line, with the Pycnogonids, Xiphosurids, Eurypterids and Arachnids as its major subdivisions, and a more varied Gnathomorph line. The latter includes the Trilobites and the Mandibulata which (*pace* Manton) comprises the Crustacea, Myriapoda and the monophyletic Insecta.

To express his phylogenetic views in taxonomic terms, Boudreaux does not flinch from introducing, between the class Insecta and its constituent orders, no fewer than thirteen different taxonomic categories and the appropriate taxa, each duly named and defined. Within this rather cumbersome framework the evolutionary affinities of the orders are discussed in ways that are sometimes widely acceptable, sometimes more controversial. The Apterygota, Palaeoptera and Exopterygota are regarded as paraphyletic and discarded, although the Entognathan apterygotes are treated as a natural group. The order Mallophaga is restricted to the Amblycera, while a sister-group, the Anoplura, is redefined to accommodate the Ischnocera, Rhynchophthirina and Siphunculata. The Plecoptera, Dermaptera and Embioptera, all with notoriously obscure affinities, are hardly more convincingly placed than in alternative classifications, and similar doubts might also apply to the Thysanoptera, Strepsiptera and Siphonaptera. The Sialoidea, Raphidioidea and Plannipennian Neuroptera all receive separate ordinal status, but the Boreidae and Micropterigidae are denied it.

In many respects the resulting scheme for the insects is not unlike those proposed by Hennig or Kristensen. What must strike a more sceptical reader, however, is the tantalising scarcity of really crucial evidence and fully convincing arguments. Despite his confident style the author does, in fact, admit the tentative nature of many phylogenetic hypotheses. Their importance lies less, perhaps, in the permanent value of the conclusions reached than in their immediate heuristic value. In this sense the views and arguments of Boudreaux and those he represents should be welcomed: they will, along with the theories of their opponents, promote the study of Arthropod morphology, development and classification, and so, one hopes, make possible one day a more widely agreed phylogeny of the Arthropoda.

R.G. Davies

R.G. Davies is Reader in Entomology at Imperial College, University of London, UK.

Semiconductor devices

Semiconductors and Electronic Devices. By A. Barl-Lev. Pp. 381. (Prentice-Hall International: London and Englewood Cliffs, New Jersey, 1979.) Hardback £11.95, $22.95; paperback £8.95, $18.95.

THIS medium-sized book written by an electrical engineer for electrical engineers embarks on the ambitious task of introducing the reader to the physical principles of semiconductor materials and leading him on to a more detailed discussion of various aspects of junction and, to a lesser extent applications of semiconductor devices. In a sense, the title is slightly misleading, implying that devices other than semiconductor ones are being treated while, in fact, the discussion of vacuum devices is minimal.

The discussion of the physical and technological principles relating to semiconductor materials covers 114 pages in 8 chapters and is necessarily sketchy, as the author admits in the preface. However, I accept that the treatment is adequate for an electrical engineering student wishing to concentrate on the practical aspects of device performance.

The next three chapters are devoted to a discussion of various aspects of junction diodes, beginning with their static characteristics and including a description of varactor diodes. The optoelectronic devices mentioned include the PIN detectors, solar cells, light emitting diodes, optic couplers and even injection lasers — the treatment here is possibly at its most sketchy in view of the different physical background required for full understanding. A more thorough chapter on the dynamic response gives a discussion of diode switching.

The treatment of transistors begins interestingly and unconventionally with a discussion of field effect transistors both of the junction and of the insulated gate types, with a brief reference to integrated circuit design. This is followed by a chapter on the amplifying stage, transistor model and the equivalent circuit which is based on the characteristics of the MOS transistor but includes mention of vacuum device and bipolar transistor characteristics, neither of which had yet been introduced. The discussion of bipolar transistors starts with the large signal model and maximum ratings and only then proceeds to small-signal low- and high-frequency models. This is a sensible arrangement and these two chapters show a rather deeper level of treatment than most of the others. One chapter deals with an introduction to integrated circuits, both bipolar and MOS, to charge-coupled devices and integrated injection logic.

The chapter on microwave devices describes very briefly the relevant contructions of the bipolar and junction FETs, gives a very cursory discussion of varactor, step-recovery and IMPATT diodes, and finishes with the transferred electron devices. The main body of the text is completed with a chapter on silicon-controlled rectifiers and triacs. There are three appendices on two-port representations, thermionic emission and vacuum tubes — the last two somewhat incongruous in the present context.

On the whole, as an introductory text for electrical engineers this is a good and interesting book. It mentions, if only briefly, most of the recent developments in semiconductor devices that the student is likely to come across. There are useful Problems at the end of each chapter. My general criticism of this strongly device-oriented text is the relative paucity of actual device characteristics — the student would have benefitted a great deal from being able to see more performance data. Also, the list of 22 references for further reading is not adequate to guide a reader who wishes to follow up specialised topics.

A.K. Jonscher

A.K. Jonscher is Professor of Solid-State Electronics at Chelsea College, University of London, UK.

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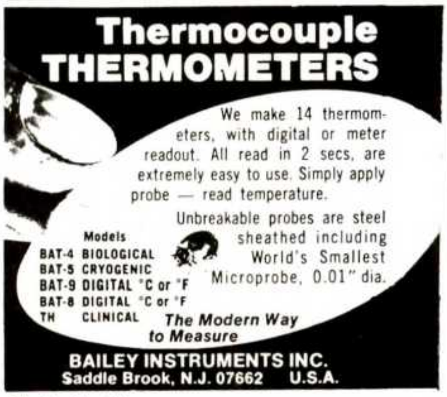
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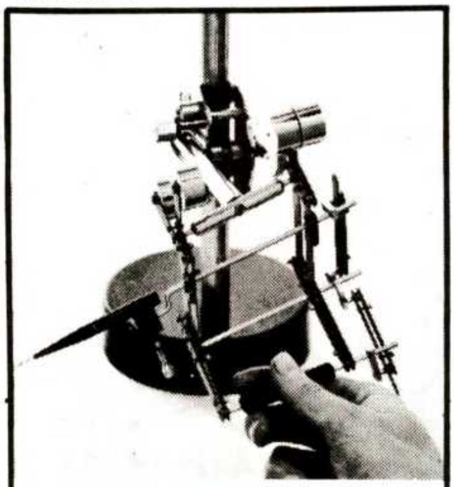
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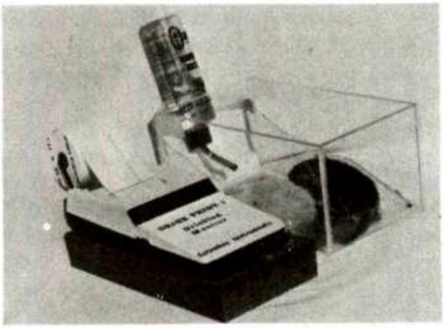
Animal drinking monitor with printer. Columbus Instruments. For measuring the trend of drinking activity of laboratory animals, the miniature drinking monitor from Columbus Instruments is equipped with periodic timer and printer. The total size including printer is 4×11×3 in. Rechargeable battery operation allows its use in any remote area. It can also be line operated. The drinking monitor (Drink-Print-1) totals the number of licks of the animal on the water dispenser and prints the totals in selected time intervals. Internal timer is crystal controlled and has four selectable intervals of 1, 2, 5 and 10 min. Printing is on ordinary office machine paper tape.
Circle No. 92 on Reader Enquiry Card.


Animal drinking monitor

Clinical products. A new 1978/80 clinical products catalogue is now available from Amersham Corporation. Products listed include RIA Kits for thyroid, obstetric/gynecology, and haematology testing as well as radioassay reagents, radiopharmaceuticals and gamma counting supplies. A technical data sheet is enclosed for each product. Circle the number shown below for a copy of the new catalogue which is supplemented by Amersham product inserts, review booklets, and monographs.
Circle No. 93 on Reader Enquiry Card.

Rhodamine conjugate series. Miles. A new series of rhodamine-conjugated products has been introduced by Miles Laboratories. The rhodamine-conjugated antisera now available are antihuman IgG (H + L), anti-rabbit IgG (H + L) and bovine crystalline albumin. Most Miles catalogue antisera are available as FITC or RITC conjugates if custom-ordered in quantities of 50 ml or more.
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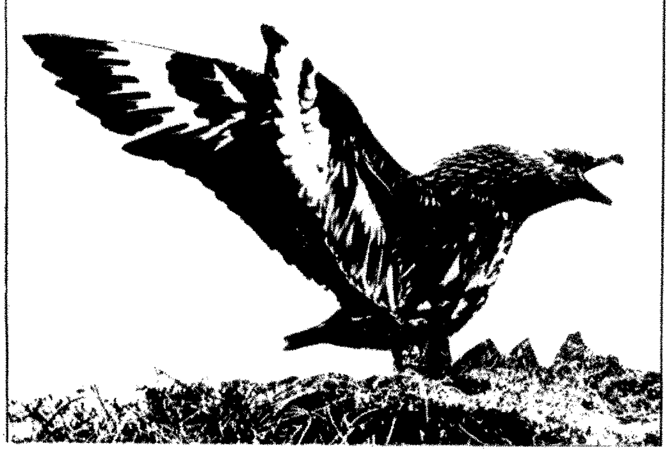

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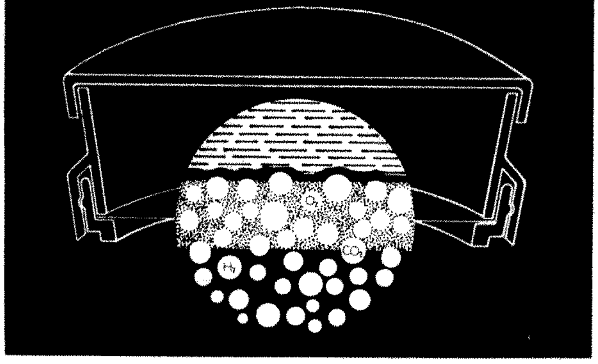
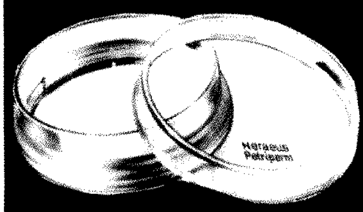
A new approach in tissue culture





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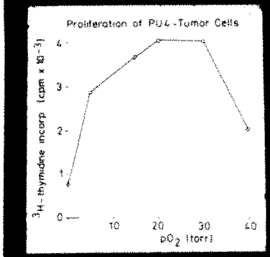

In the intact animal, the pH, and the partial pressure of both oxygen and CO_2 of the cell environment are under total biochemical and physiological control. This state is not even approximated by available techniques of monolayer cell culture in conventional tissue culture ware. Continuously changing gradients of these parameters in the micro-environment of cultured cells will obscure the biological significance of culture dependent phenomena. PETRIPERM is the prerequisite for the precise control of pO_2, pCO_2 and pH in the cellular environment as gases reach the cultured cells directly through the highly gas permeable 25 μm thick membrane.

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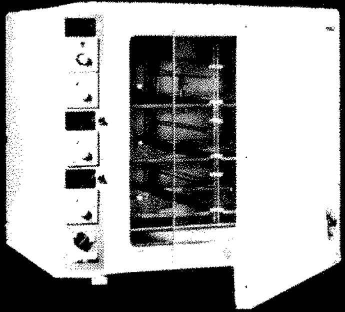

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Specific endopeptidase for sequence analysis. Pierce. Submaxillaris protease, an arginyl-specific endopeptidase, is now available from Pierce. Isolated from the submaxillary glands of mice, it is reported to demonstrate greater specificity than Clostripain towards the arginyl peptide bond. Use of submaxillaris protease promises to be a most effective approach to obtaining arginyl terminating peptides for sequence analysis. No modification of lysine side chains is necessary to direct cleavage to arginyl loci in peptide chains. The protease is supplied lyophilised and salt-free in 100-unit packages.
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Freeze-drying station. FTS Systems. Dynamic testing of heat-sensitive biologicals is performed in twin 14-freeze-dryers which are coupled to a programmable, recording console. Either unit may operate independently or simultaneously for comparison-testing the effects of vacuum and heat input to products while all variables are recorded on the control console. Special features include; low mass, fully flooded, stainless steel product-shelves with a product-freezing and heating facility. Product trays are locked down to make firm contact with the temperature-controlled shelves; materials with high acid resistance are used in the ice condensing system. Gas sterilisation, full micrometer control over vacuum and sterile air inlets, and special accessories to match individual product-drying requirements.
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Vacuum/compressor pump. The Edwards D.1 diaphragm pump achieves vacuum better than 200 mbar and delivery pressure of 4.2 kgf per sq cm, and is completely oil-free in operation. The pump weighs only 7.7 kg and its noise level is only 50 dB(A). Complete with carrying handle, the D.1 is very reliable and simple to maintain. The main shaft is of constant diameter, eliminating potential failure points, and simple disposable filters can be rapidly changed. The pump design makes subsequent incorrect assembly impossible. Inlet and exhaust ports are both in the head, which can be rotated through 180° to reverse their positions.
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Micromanipulator. Singer. The purpose of the Singer Microforge is to make easily from glass, micropipettes, needles both straight and curved, loops etc. The glass and heating element are viewed through a binocular eyepiece microscope with fine focusing. The glass and platinum heating element are held in fully adjustable and rotatable holders and the special feature of the instrument is that these holders are manipulated by two joysticks that move the work in the focal plane. This design advance makes possible, with little practice, the performance of intricate and complicated movements. The microscope and joysticks and other controls are conveniently mounted on a single rigid chassis within the instrument case. The heating element is thyristor controlled, and the element temperature is smoothly and finely adjusted by a large, conveniently placed knob.
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Nick translation system (^{32}P). New England Nuclear announce the availability of their new nick translation system dCTP, $[\alpha\text{-}^{32}P]$—for performing nick translation of DNA. The resulting labelled DNA product is suitable for use in kinetic studies (as a substrate for restriction enzyme digestion) or probe hybridisation methodology. The eight-vial system includes all necessary components for approximately 20 reactions.
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Portable data logging systems. Microdata. The M1600L from Microdata is a multi-channel data logger recording on a computer-compatible magnetic tape cartridge. It is capable of interfacing with any types of analogue or digital transducer by the use of a separate plug-in signal conditioning module for each channel. The basic instrument can accommodate up to 20 modules in its own case (making a 20-channel capacity), with provision for expansion to 100 channels by the use of add-on units. An important feature in the load monitoring application is the M1600L's self-contained portability. It requires no external power supply, and is capable of operating for long periods without attention from its internal rechargeable battery.
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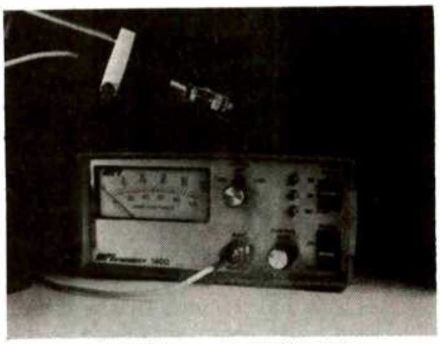

The W-P Instruments nanolitre pump

Programmable computerised image analysers. The new Quantimet System 23 from Cambridge Instrument Company, is a fully programmable, computerised image analyser that does not need a programmer. The new System 23 enables the operator to write comprehensive application-specific programs for his specimens without knowledge of programming languages or computer skills. A unique interactive image analysis keyboard, together with a powerful and versatile software operating system makes it all possible. Once a program is written, it can be stored on a high-speed magnetic disk enabling immediate use whenever required. The System 23 provides an additional capability for those operators who can program in FORTRAN and BASIC.

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Nanolitre pump. W-P Instruments. A small, light-weight instrument from W-P Instruments for micro-perfusion, chemical titration, and bioassay use can be brought directly to the site at which fluids will be delivered or removed at rates from below 5 nl $\min^{-1}$ to 100 nl $\min^{-1}$. Fluid delivery is effected by a glass micropipette drawn to a tip diameter in the 3–10-μm range. The pump body contains a precision d.c. miniature motor. Motor speed is kept constant by electronic feedback control. Electrolytes, drugs, dyes and other fluids may be displaced into or out of the pump in extremely small volumes and with variable rates. It is designed to be mounted in any standard micromanipulator.

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Micro-computer. IN/US Service Corporation. A new four-page brochure published by IN/US Service Corporation describes the Model 5000 Micro-Computer, which can turn a terminal into a computer. The Model 5000 may be used for many applications in bio-medical laboratories and the brochure gives particular emphasis on liquid scintillation and gamma counting. Simplicity of programming is a feature of the Model 5000. Although the Model 5000 has been designed to be user-programmed, it is delivered with turn-key programs for a variety of counting and statistical applications.

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HPLC peak collector. Gilson. A new peak selective fractionator collecting only peaks has been announced by Gilson Medical Electronics. The peak collector connects to UV, visible or fluorescence detectors to perform high flow preparative collection or repeat sample injection for HPLC. Peaks are identified by the threshold level and by increasing or decreasing concentration. Peak identification circuitry controls the mobile indexing head, collecting one peak per tube and up to 15 peaks per sample injection.

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Fundamentals of Data Base Systems

S. M. Deen

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Programming and Problem Solving in Algol 68

Andrew Colin

An introduction to the art of programming and a grounding in the specific language of Algol 68. The author provides much background information about the environment in which computers operate, introduces algorithms such as sorting and searching, and gives many examples throughout.

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Analysis and Design of Sequential Digital Systems

L. F. Lind and J. C. C. Nelson

Begins by introducing logic design and several concepts to be used later in the work, and goes on to examine combinational-logic design (developing and using the Karnaugh map) and sequential design, both synchronous and asynchronous. Topics covered include stable and unstable states, excitation and flow maps, input and output maps, static, dynamic and essential hazards, and merging and state assignment.

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Established titles

Microprogramming

Guy Boulaye

Edited by P. A. Beaven

A concise introduction to microprogramming for students of computer sciences, computer technology and (digital) electronic engineering.

1975, £3.95 paperback.

Experiments with Operational Amplifiers: Learning by Doing

G. B. Clayton

Covers a range of practical operational amplifier applications. The author contends that the quickest way for the non-specialist to learn about amplifiers is to use them in working circuits, and aims to give a practical understanding of the principles behind each application.

1975, £2.95 paperback.

Automata

D. R. Hopkin and Barbara Moss

Macmillan Computer Science series

A self-contained account of the theory of finite and infinite automata, with special reference to those machines which model aspects of computation. The text assumes no previous knowledge of logic or formal arithmetic, and develops the theory through a wide variety of worked examples.

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in plant physiology and biochemistry, available in the above Department. Candidates should have a good Honours Degree in Botany, Biology or Biochemistry and a PhD in Plant Physiology or Biochemistry. The posts are available in a group working on the regulation of C_3, intermediate and C_4 metabolism in the photosynthesis of crop plants with particular reference to temperature. The work is concerned with analysis of metabolite fluxes and the enzymology of rate-limiting steps in photosynthetic assimilation of CO_2. The research group is directed by Professor H W Woolhouse and is financed by the ARC. The posts are tenable for up to 5 years from October 1979.

Salary on the IA scale for Research and Analogous Staff (£4,333 to £7,521) (under review).

Further information may be obtained from Professor H W Woolhouse, Department of Plant Sciences (Baines Wing), University of Leeds, Leeds LS2 9JT.

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference number 53/12. Closing date for applications 30 September 1979. 3090(A)

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from suitably qualified graduates. The Area laboratory at The Leicester Royal Infirmary offers wide experience of automated and non-automated techniques. New laboratory accommodation is due to be opened shortly.

Further details may be obtained from:
Dr T.J. Davies, Consultant Chemical Pathologist, telephone Leicester (0533) 541414 ext. 5574 or 331 who can arrange visits to the laboratory.

Applications, with curriculum vitae and names of two referees should be sent to the Hospital Administrator, The Leicester Royal Infirmary, Infirmary Square, Leicester.

Closing date for receipt of applications: 17th September, 1979.

Leicestershire Health Service 3140(A)

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A number of studentships for study to PhD are also available. 3095(A)

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The Department of Geology in the School of Earth Sciences invites applications for a faculty position beginning September, 1980. Areas of interest include (but are not limited to) geochemical processes in the crust and mantle of the earth including isotope geochemistry, thermodynamics, kinetics, element distribution and phase equilibria studies. Teaching responsibilities might include graduate and undergraduate courses in geochemistry and igneous petrology. Level of appointment is open, but we are particularly interested in a creative young person, with a well-established record in research.

Applications should include (1) a resume of education, professional experience, and published research; (2) a brief statement of plans for future research, and (3) the names of 4 referees. Send to: Prof. J.G. Liou, Department of Geology, Stanford University, Stanford, CA 94305, by December 1st, 1979. Stanford University is an equal-opportunity/affirmative-action employer. W329(A)

SIMON FRASER UNIVERISITY

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DEPARTMENT OF BIOLOGICAL SCIENCES

DIRECTOR OF MASTER OF PEST MANAGEMENT PROGRAM

Applications are invited for the vacant position of Director of the Master of Pest Management (MPM) program and related activities at Simon Fraser University.

The appointment will be at the rank of Associate Professor or Professor, with level and salary commensurate with training and experience. The successful candidate should have an established professional and academic reputation in pest management and a current research program in at least one aspect of pestology. Responsibilities will include the development and organisation of the MPM program as well as research and teaching in the individual's area of expertise. The position is available as of 1 September, 1979, subject to budgetary approval. The current salary range for the Associate Professor and Professor rank is, respectively, $25,636 to $41,018 and $31,552 to $55,216 per annum.

Applications, including an up-to-date curriculum vitae, copies of representative recent publications and the names of at least three referees, should be sent to: Dr M Mackauer, Chairman, Department of Biological Sciences, Simon Fraser University, Burnaby, BC, Canada, V5A 1S6 from whom further information may be obtained. Applications will be received until 31 October, 1979 or until the position is filled. W341(A)

UNIVERSITY OF NEWCASTLE UPON TYNE

CHAIR OF THEORETICAL PHYSICS

Applications are invited for the Chair of Theoretical Physics in the School of Physics from candidates whose teaching and research interests lie broadly within that field. It will be an advantage if an applicant is a theorist with primary interests related to areas of activity in one of the School of Physics' three departments. The appointment, which is available from the 1st October 1980, carries with it the Headship of the Department of Theoretical Physics for an initial period of five years. Salary will be in the professional range.

Further particulars may be obtained from the Deputy Registrar, University of Newcastle upon Tyne, 6 Kensington Terrace, Newcastle upon Tyne, NE1 7RU with whom applications (15 copies) giving the names of three referees, should be lodged not later than 31st October 1979. (Applicants from outside the British Isles may submit one copy only.) 3082(A)

SCHOOL OF PHYSICAL SCIENCES POSTDOCTORAL RESEARCH ASSISTANTS IN CHEMISTRY

Applications are invited for two S.R.C. sponsored research posts, each tenable for two years from 1st October, 1979 (or as soon as possible thereafter).

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The second appointment (quote Ref: 79/64), which will be under the supervision of Dr A.C. Knipe, will involve investigation of 'Organic Reaction Mechanisms', with particular emphasis on novel elimination reactions of sulphonamide derivatives and the reactivity of small-ring systems.

The commencing salary for each post is £4,910 per annum (with FSSU/USS).

Further particulars may be obtained from the Registrar, The New University of Ulster, Coleraine, Northern Ireland, BT52 1SA (quoting appropriate reference number) to whom applications including the names and addresses of three referees should be sent as soon as possible.

3085(A)

POSTDOCTORAL FELLOW or RESEARCH ASSOCIATE to participate in studies of molecular events in RBC differentiation and development, including studies of nucleic acid synthesis, production of globin and non-globin proteins, and iron metabolism. Applicant should have experience with biochemical methods for analysis of nucleic acids *or* protein synthesis. Salary range $11,500 to $14,000 depending upon experience. Send curriculum vitae, including 3 references to: Dr Joseph A. Grasso, Department of Anatomy, University of Connecticut Health Center, Farmington, Connecticut 06032

W348(A)

THE UNIVERSITY OF LEEDS

DEPARTMENT OF PLANT SCIENCES

Applications are invited for a post of

RESEARCH ASSISTANT

in plant physiology biochemistry, available in the above Department. Candidates should have a good Honours Degree in Botany, Biology or Biochemistry; the opportunity will exist for the holder of the post to register for a higher degree. The post is available in a group working on the regulation of photosynthesis in crop plants with particular reference to effects of temperature. The work is concerned with analysis of metabolite fluxes and the enzymology of rate limiting steps in the photosynthetic assimilation of CO_2. The research group is directed by Professor H W Woolhouse and financed by the ARC. The post is tenable for up to 5 years from October 1979.

Salary on the IB scale for Research and Analogous Staff (£3,775 to £5,488) (under review).

Further information may be obtained from Professor H W Woolhouse, Department of Plant Sciences (Baines Wing), University of Leeds, Leeds LS2 9JT.

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference number 53/13. Closing date for applications 20 September 1979.

3091(A)

CITY OF BIRMINGHAM POLYTECHNIC

Faculty of Engineering and Science

DEPARTMENT OF SCIENCE

LECTURER II/SENIOR LECTURER in BIOMEDICAL SCIENCES (PHYSIOLOGY)

required to contribute to the teaching of courses in Medical Laboratory sciences, Speech Therapy and Biological Science. Candidates should have suitable academic qualifications and have a keen interest in contributing to the development of research activity in the appropriate field of Human Physiology but an ability to contribute to the teaching of Cell Biology and/or Genetics would be an advantage.

Salary Scales:

SENIOR LECTURER: £6,597 to £7,701 (bar) £8,253

LECTURER II: £4,470 to £7,149.

Further details and application forms (to be returned by 21st September 1979) from: The Personnel Officer, City of Birmingham Polytechnic, Perry Barr, Birmingham B42 2SU. (021-356 6911, Ext. 217).

3093(A)

ASSISTANT OR ASSOCIATE Professor of Entomology to conduct systematics research and teach principles of systematics and insect classification. PhD required. Submit curriculum vitae, transcripts, description of research experience and plans, reprints of publications, dissertation summary, summary of teaching capabilities, names of 3 referees to A.A. Grigarick, Chairperson, Search Committee, Department of Entomology, University of California, Davis, CA 95616 by October 1, 1979. An Equal Opportunity /Affirmative Action Employer.

W335(A)

UNIVERSITY COLLEGE OF WALES

Welsh Plant Breeding Station

Applications are invited for the post of

HEAD OF SEED PRODUCTION DEPARTMENT

The Department has responsibility for production and processing of seed stocks for Station and National List trials and for Pre-basic seed stocks of Station-bred varieties. Its research programme is concerned with the development of effective seed production techniques, with emphasis on seed yield and quality. An important aspect of the post is liaison with public and private organisations concerned with seed production, testing and certification.

The candidate should have a good Honours degree in agricultural or biological science, with considerable experience in plant breeding and/or seed production.

The post is in the PSO range at a salary of £7,534 to £9,646 per annum (from 1 January 1980, £8,513 to £11,343). *Non-contributory* superannuation scheme but male employees contribute 1½% towards family benefits.

Further particulars and application forms are obtainable from The Secretary, Welsh Plant Breeding Station, Plas Gogerddan, Nr Aberystwyth, Dyfed, SY23 3EB. Closing date Monday, 1 October 1979. Please quote reference 45.

3136(A)

Physicist/ Physical Chemist

BP has a vacancy for a physicist or physical chemist in its Exploration and Production Division at the BP Research Centre, Sunbury-on-Thames.

The work is within a small but expanding group engaged in research into the physical basis of oil recovery. Applicants should have experience in the measurement of the thermodynamic or transport properties of fluids, preferably to Ph.D. level, together with an interest in the Earth Sciences. You will be expected to make a contribution to the philosophy and direction of the project work and have the ability to translate oil field problems into physical and mathematical models.

Salary will be dependent on qualifications and experience and assistance will be given with relocation expenses where appropriate. You will have the benefit of a non-contributory pension scheme, subsidised lunches and an excellent sports and social club.

If you would like to be considered for this post, please write giving details of your career, qualifications and experience to: Mrs. L. Fuller, Personnel, BP Research Centre, Chertsey Road, Sunbury-on-Thames, Middlesex TW16 7LN. The closing date for application is Monday 17th September 1979.

BP Research Centre
Sunbury on Thames

(3108)A

UNIVERSITY OF NEWCASTLE UPON TYNE

Department of Geology

DEMONSTRATORSHIP IN PETROLOGY

Applications are invited for a full-time demonstratorship for three years from 1st October 1979 or as soon after that as can be arranged. The successful candidate will teach the petrology, geochemistry and mineralogy of igneous and metamorphic rocks, and hydrothermal ore deposits, as well as some basic geology. Postgraduate experience in this general area is a necessary pre-requisite. Opportunities will be available for developing new courses. Research is strongly encouraged.

Salary will be at an appropriate point on the Grade 1B (bar) scale £3,775 to £5,488 per annum according to age, qualifications and experience. Membership of the appropriate University superannuation scheme will be required.

Further information can be obtained from Professor J.R. Cann, Department of Geology, The University, Newcastle upon Tyne NE1 7RU, with whom applications (2 copies), giving the names and addresses of three referees should be lodged as soon as possible and in any case not later than 14th September, 1979.

3123(A)

MEDICAL RESEARCH COUNCIL

DEVELOPMENTAL NEUROBIOLOGY UNIT

INSTITUTE OF NEUROLOGY

Applications are invited from scientists with a few years of postdoctorate experience for a 5 year limited term position. The research primarily concerns the characterisation of neurotransmitter systems of the cerebellum, including studies on isolated cell types and cultured cells from the cerebellum, and also involves collaborative work with morphologists in the Unit. Previous experience in biochemical neuropharmacology, membrane biochemistry and/or cell biology would be an advantage. Salary on MRC Non-clinical Scientific Staff scales depending on age and experience (Grade II, £4,232 to £6,627 or Grade I £6,757 to 8,452 plus £502 London Allowance).

Applications, together with full curriculum vitae and the names of two professional referees should be sent to Dr R. Balazs, MRC Developmental Neurobiology Unit, Institute of Neurology, 33 John's Mews, London WC1 2NS.

3129(A)

Public Health Laboratory Service

PHLS Centre for Applied Microbiology & Research

Diagnostic Reagents Laboratory

Basic Grade Microbiologist

A research biochemist with experience in enzymology and peptide chemistry is required. The position will involve research on the development and characterisation of new proteins/enzymes for use in diagnostic assays; the development of new assay systems for the detection of a wide range of compounds, including human metabolites and the detection and analysis of peptides, in particular those in serum.

Salary will be in the range £3,486 to £5,592 increasing to £3,807 to £6,075 on 1.1.80. NHS terms and conditions of service.

The post may be discussed informally by contacting Dr R. Sherwood or Dr A. Atkinson, Diagnostic Reagents Laboratory. Tel. Idmiston (0980) 610391, exts 435/393.

Applications including curriculum vitae and names and addresses of two academic referees to Mrs M. Bushby, Personnel Officer, CAMR, Porton Down, Salisbury, Wilts SP4 0JG. Tel: 0980-610391, ext 298. Closing date 21 September, 1979.

Special Pathogens Reference Laboratory

Principal Grade Microbiologist

(£7,995 to £10,458 increasing to £9,129 to £12,306 on 1.1.80)

A Principal Grade Microbiologist is required by the Special Pathogens Reference Laboratory to institute and develop a programme of biochemical and immunochemical investigations of viral pathogens of interest to the laboratory. At present these include Lassa, Marburg and Ebola viruses and certain arboviruses. The appointee will be responsible to the Director of the laboratory, and will be expected to share in routine laboratory investigations. He/she will lead a small research team.

Qualifications: A PhD obtained in the field of viral biochemistry together with at least 3 years of post-doctoral research experience preferably with work on human pathogens. It is envisaged that the appointee will already have experience of the procedures required for the growth and purification of viruses together with a knowledge of methods for the separation of viral proteins. Previous experience in the serological and immunochemical analysis of viral protein components would be of advantage.

The Laboratory Director, Dr D. Simpson, would be pleased to discuss the post informally with interested candidates, tel. 0980-610391.

Applications including Curriculum Vitae and names and addresses of two academic referees to be submitted to Mr R.S. Sawyer, PHLS HQ, 61 Colindale Avenue, London NW9 5EQ. Tel 01-200 1295. Closing date 5 October 1979.

PHLS

3103(A)

To place your advertisement in these pages
Phone: 01-831-6901

LABORATORY MANAGER

Grade 10 (£7,464 – £8,040 + £312 Supplement)

(Salaries under review)

The Welsh Water Authority has five Area Laboratories which provide a complete analytical service (chemical, biological and microbiological) across Wales.

A vacancy exists for the position of Laboratory Manager at the Nash Laboratory situated outside Newport, Gwent. The Laboratory Manager is an outposted member of the Directorate of Scientific Services and will report to the Laboratory Controller. Applicants must be enthusiastic and well qualified Scientists who have had experience in managing a multi-disciplinary laboratory or group of Scientists. Experience in one or more aspects of the water cycle would be a distinct advantage.

The Laboratory has a staff of about 30 and consists of a number of groups covering the various disciplines. A high degree of automated analysis is in use.

The terms and conditions of employment are as laid down by the National Joint Council for Water Service Staffs. Relocation expenses are payable in appropriate cases and a Regular User Car Allowance is attached to this post.

Application forms may be obtained from:

Assistant Personnel Officer (Ref 2L002)
Welsh Water Authority Cambrian Way
Brecon Powys, LD3 7HP
Tel: Brecon (0874) 3181 Extension 373
Closing Date for applications: 24th September 1979

WELSH WATER AUTHORITY
AWDURDOD DWR CYMRU

3141(A)

TECHNICIAN IN PHYSIOLOGICAL PHYCHOLOGY required in the Department of Psychology to assist in teaching and research laboratories. Duties will involve work with animals. Previous experience with histological, biochemical, physiological or pharmacological procedures will be advantageous. Applicants should have HNC Biology or equivalent. Salary Scale £3,222 to £3,708 p.a. Application form from Assistant Secretary, Personnel Office, University of Birmingham, PO Box 363, Birmingham B15 2TT. Ref. 121/c/372. 3112(A)

THE UNIVERSITY OF SHEFFIELD

ACADEMIC DIVISION OF MEDICINE

LECTURESHIP IN PHARMACOLOGY AND THERAPEUTICS

Applications are invited from men and women for the above post in the University Department of Pharmacology and Therapeutics. Duties include teaching experimental pharmacology to students of medicine, dentistry and pure science. In addition to teaching and administrative duties, the undertaking of original research in some aspect of experimental pharmacology is required. Initial salary in range £4,333 to £5,199 a year on scale rising to £8,992 a year. Those interested should in the first place contact Professor H.F. Woods, Department of Therapeutics, the Hallamshire Hospital, Sheffield S10 2JF. Tel. 0742 26484 ext. 2475. Further particulars from the Registrar and Secretary, the University, Sheffield S10 2TN to whom applications (8 copies) should be sent by 1 October 1979.

Quote ref: R 355/G. 3092(A)

TEMPLE UNIVERSITY, Department Biology, Postdoctoral Position. A tw year postdoctoral position in mammalia developmental genetics and reproducti biology is available immediately. Initi salary is $10,500 p.a. plus fringe benefit Applicants should send, as soon possible, a curriculum vitae and names of referees to Dr Nina Hillman, Temp University, Biology Departmen Philadelphia, Pennsylvania 19122.

W340(A)

UNIVERSITY COLLEGE CARDIFF

Applications are invited for an

SRC POST DOCTORAL FELLOWSHIP

available for suitably qualified candidat to work with Dr J.W.T. Wimpenny in t Department of Microbiology, c quantitative aspects of the growth microorganisms in solute gradients. T successful applicant will join a grou investigating the biology of three differe diffusion systems, and will be responsib for generating mathematical mode capable of defining and predicting t growth and interactions of organisms solute gradients. Applicants having suitable mathematical background a anxious to gain some experience in biological discipline are sought.

The appointment is tenable for 2 years a starting salary of £4,333 (under review

Applications (2 copies) includi curriculum vitae and the names a addresses of two referees should forwarded to the Vice-Princip (Administration) and Registrar, Universi College, PO Box 78, Cardiff CF1 1X. Closing date 1st October 1979. Referen 1891. 3111(A)

Southampton THE UNIVERSITY

DEPARTMENT OF BIOLOGY

A research Assistant is required to work with Dr. P. Thorogood on an MRC-funded project concerned with the role of cell and tissue interactions in the differentiation of neural crest cells. Applicants should have a good honours degree in one of the biological sciences, a strong interest in developmental biology and one or two years experience in a research laboratory. Expertise in cell and tissue culture techniques would be an advantage. The post is tenable for three years commencing 1st October, 1979 or as soon as possible after that date, and the initial salary will be up to £4,055 per annum. Registration for a higher degree will be considered. Applications, together with a curriculum vitae and the names of two referees should be sent as soon as possible to Dr. P. Thorogood, Department of Biology, Medical and Biological Sciences Building, Bassett Crescent East, Southampton SO9 3TU. Please quote reference number 1602/R/N.

3121(A)

WELSH NATIONAL SCHOOL OF MEDICINE

(University of Wales)

DEPARTMENT OF HAEMATOLOGY

TECHNICIAN (ELECTRON MICROSCOPY)

A technician experienced in electron microscopy is required to carry out routine processing of blood, bone marrow cells and other tissues. The person appointed will have the opportunity to take part in the department's research programme in erythropoiesis and iron metabolism and to develop an individual interest. The School's electron microscope unit is now adding a Philips 400 STEM to its equipment and this will be operational early in 1980.

Salary for State Registered technicians will be on the scale for medical laboratory technical staff (£3,768 to £5,391 per annum) or that for Senior Medical Laboratory technical staff (£5,016 to £6,585 per annum) as appropriate. The department may be visited by arrangement with Professor A. Jacobs (Cardiff 755944 Ext. 2375).

Application forms available (quoting ref. No.H24/1) from the Registrar and Secretary, Welsh National School of Medicine, Heath Park, Cardiff CF4 4XN to whom they should be returned 2 weeks from the date of appearance of this advertisement. 3102(A)

UNIVERSITY OF BIRMINGHAM

DEPARTMENT OF GEOLOGICAL SCIENCES

LECTURESHIP

Applications are invited for a lectureship with special interest in sedimentology. This is a newly established post and will be tenable from 1st October 1979. Salary will be in the range £4,333 to £8,992 (October Scales) plus superannuation. Starting salary will depend on age, experience and qualifications.

Further particulars are available from the Assistant Registrar, Science and Engineering, University of Birmingham PO Box 363, Birmingham B15 2TT, to whom applications (3 copies) including full curriculum vitae and naming three referees should be sent by Friday 21st September 1979.

Pleae Quote ref: NG5. 3086(A)

Physicists

For Rutherford Laboratory's High Energy Physics Programme

The Rutherford Laboratory is one of the Science Research Council's leading centres. The Laboratory maintains an active experimental programme based principally on Accelerators at the European Organisation for Nuclear Research (CERN) in Geneva and at the Deutsches Electronen-Synchrotron (DESY) in Hamburg.

Their High Energy Physics Division now has two vacancies for young Experimental High Energy Physicists at either Senior Scientific Officer or Higher Scientific Officer level.

Applicants should be post-doctoral physicists with experience in the formation, running and analysis of high energy physics experiments.

A certain amount of time would be spent abroad at CERN or DESY, and candidates will be required to work in collaboration with Physicists, both from British Universities and from abroad.

Depending on qualifications and experience, salaries will be in the range:-

Depending on qualifications and experience, salaries will be in the range: – Senior Scientific Officer: £6,290-£8,415 (£6,780-£9,315 from 1st. Jan. 1980). Higher Scientific Officer: £5,060-£6,650 (£5,455-£7,210 from 1st. Jan. 1980).

There is a contributory pension scheme, generous leave and sick leave allowances.

Application forms are available from Jane Griffiths of the Personnel Group. Telephone her on Abingdon (0235) 21900 Ext. 510, or write, quoting reference VN822 Rutherford Laboratory, Chilton, DIDCOT, Oxon OX11 0QX.

Closing date for applications 21st September 1979.

3097(A)

POST-DOCTORAL POSITION IN ELECTRON DIFFRACTION CRYSTAL STRUCTURE ANALYSIS

A position is available after November 1, 1979 for research on the development and application of electron diffraction techniques for organic crystal structure analysis (small molecules and linear polymers). Quantitative use of transmission e.d. data will include application fo the direct phasing technique used in X-ray crystallography with concomitant evaluation of the deleterious influence of n-beam dynamical interactions and crystal deformations on the success of such structural analysis. The applicant should have a background in diffraction physics and crystallography and should be able to operate an electron microscope.

Appointment initially for one year; probably renewable. Address applications with 3 references to D.L. Dorset, Molecular Biophysics Department, Medical Foundation of Buffalo, Inc., 73 High Street, Buffalo, New York 14203, U.S.A. W334(A)

FLOUR MILLING AND BAKING RESEARCH ASSOCIATION

ORGANIC CHEMIST

The Flour Milling and Baking Research Association carries out a wide range of research and service work for the Milling and Baking Industries and holds a number of research contracts from the Ministry of Agriculture, Fisheries & Food. The laboratories are situated in an attractive semi-rural area on the edge of the Chilterns with ready access to London.

A **GRADUATE ORGANIC CHEMIST** is required to join a small team studying the composition and properties of cereal bran and germ. The successful candidate will initially be involved in the development and application of methods of analysis of complex carbohydrates.

The post is permanent and superannuated, and will be graded Scientific Officer (£3,194 to £4,767) or Higher Scientific Officer (£4,530 to £5,904), according to qualifications and experience.

Further details and an application form may be obtained from Professor Brian Spencer, Flour Milling and Baking Research Association, Chorleywood, Rickmansworth, Herts. WD3 5SH. Tel: Chorleywood (STD 092 78) 4111. 3135(A)

Horticultural Centre Loughgall Higher Scientific Officer

£4,727 – £6,211

(£5,097 – £6,737 from 1st January 1980)

Applications are invited for a pensionable post in the Horticultural Centre of the Department of Agriculture, situated at Loughgall, Co Armagh.

DUTIES

The person appointed will be responsible for chemical and biological services associated with experimental work within horticulture. The duties will also include the direction and development of the recently constructed centralised laboratory unit.

QUALIFICATIONS

Applicants must be under 30 years of age at 31 December 1979 and possess a first or second class Honours Degree in Chemistry, Biochemistry or an appropriate relevant subject and have at least 2 years' appropriate post graduate experience.

A knowledge of commercial horticulture is desirable but not essential.

Exceptionally, applications may be considered from candidates over the age limits who have specialised experience.

The Civil Service Commissioners may decide to interview only those applicants who appear from the information available (including level of academic attainment and experience) to be best qualified.

SALARY

Higher Scientific Officer £4,727 – £6,211.

Starting salary will be related to qualifications and experience.

There are prospects of promotions to Senior Scientific Officer (£5,876 – £7,864) and Principal Scientific Officer (£7,534 – £9,646). The aforementioned salary scales are subject to review from 1st January 1980.

Please write or telephone for an application form quoting job reference SB 267/79/N to the Civil Service Commission, Rosepark House, Upper Newtownards Road, Belfast BT4 3NR (telephone Dundonald 4585 ext 256). Completed forms must be returned to arrive not later than 20th September 1979.

3128(A)

NORTHERN IRELAND CIVIL SERVICE

UNIVERSITY OF OXFORD
in association with
Lady Margaret Hall

UNIVERSITY LECTURESHIP IN INORGANIC CHEMISTRY

Applications are invited for the above post to be filled from 1 October 1980 or earlier if possible. Stipend according to age on the scale £4,232 to £9,000. The successful candidate may be offered a Tutorial Fellowship in Inorganic Chemistry by Lady Margaret Hall associated with a lecturership at Brasenose College, both college appointments being open to men and women.

Details of the University and college appointments may be obtained from Professor J.B. Goodenough, Inorganic Chemistry Laboratory, South Parks Road, Oxford, OX1 3QR, to whom applications (6 copies) should be sent by *1 November 1979.* 3114(A)

THE UNIVERSITY OF MANCHESTER. Research Technician (grade 5) required to work in the Department of Anatomy. Applications are invited for the post of research technician to work in the area of experimental neurology. Applicants must hold HNC or equivalent and have at least 8 years' laboratory experience. Competence in a wide range of modern techniques in histology and microscopy including autoradiography is essential. Previous experience in research work is required and a working knowledge of the use of neuroanatomical, neurophysiological or neuropharmacological research techniques would be a considerable advantage. Salary on the interim scale £3,700 to £4,320 p.a. together with further comparability payments and subject to overall review as from 1st October 1979. Applications giving details of age, qualifications, experience and the names of two referees should be sent to Professor P.F. Harris, Department of Anatomy, Medical School, University of Manchester, Manchester M13 9PT, by 30th September 1979. 3109(A)

ROYAL POSTGRADUATE MEDICAL SCHOOL
DEPARTMENT OF CLINICAL PHARMACOLOGY

SENIOR RESEARCH OFFICER (Postdoctoral)

with experience of cell culture and protein purification is required for two years to join a collaborative project between the Central Toxicology and Coporate Laboratories of ICI Ltd. and the Department of Clinical Pharmacology, Royal Postgraduate Medical School. The successful applicant will join a multidisciplinary team working on the isolation and characterisation of multiple forms of cytochrome P-450 from human and animal tissue.

Salary on range 1A for non clinical research staff.

Application forms and further particulars may be obtained from the Personnel Office, Royal Postgraduate Medical School, 150 Du Cane Road, London W12 0HS quoting reference number 20/ICI/N. 3094(A)

UNIVERSITY OF BRISTOL
BIOMECHANICS

Assistance is required for a project between the University and the S.W. Regional Hospitals to investigate the mechanics of total hip replacement. The experimental approach will initially involve strain gauge instrumentation of the femur and the femoral prosthesis in an animal model in vivo. The appointment will be as

RESEARCH ASSISTANT 1A

Preference will be given to those with a PhD, or other post graduate experience.

Salary from £4,232 p.a. depending on experience. (Scale subject to revision with effect from 1st October, 1979).

Applications, together with a c.v. and the names of two referees to Dr L.E. Lanyon, School of Veterinary Science, Park Row, Bristol BS1 5LS by September 22nd. 3101(A)

DEVELOPMENT BIOLOGIST — an assistant professorship (tenure track) is available starting September 1980. Primary duties include teaching and research in molecular and cellular aspects of development. Applicants should send curriculum vitae, reprints, and three letters of recommendation to: Development Search Committee Chairperson Division of Biological Sciences, University of Michigan, Ann Arbor, Michigan 48109, by November 10, 1979. A non-discriminatory affirmative action employer. W343(A)

UNIVERSITY OF MALAYA

Applications are invited for the following post:

FACULTY OF SCIENCE

Post of Lecturer in the Department of Geology in the field of Structural Geology or Palaeontology.

Qualification: Candidates must possess at least a Master's degree in the required field; or PhD in the required field.

Salary scales (approx. stg. equiv.): Lecturer, £3,602 × 168 — 3,939/4107 × 168—4,443/4780 × 280 — 6,182/Review Point — £6,462 × 336 — 6,799.

Further particulars and application forms are obtainable from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

The closing date for the receipt of applications is 30 September, 1979.

3106(A)

UNIVERSITY OF LONDON KING'S COLLEGE
DEPARTMENT OF GEOLOGY

Applications are invited for the post of

TUTORIAL STUDENT

in the Department of Geology, tenable from 1st October, 1979 until 30th September, 1980 in the first instance but renewable for two further years. Applicants must be, or expect to be, graduates and will be required to read for a higher degree by research.

The award amounts to £2,500 per annum with free tuition in the College, in return for a limited amount of tutorial or demonstration assistance in the Department.

Application forms and conditions of appointment from: The Assistant Registrar, King's College, Strand, London WC2R 2LS, to whom completed applications should be returned as soon as possible. 3107(A)

UNIVERSITY OF MANCHESTER

RESEARCH ASSOCIATE IN MEDICAL BIOCHEMISTRY

Applictions invited from post-doctoral candidates for this post (available for three years) to work on glycoproteins and proteoglycans in collagenous structures of blood vessels. Some experience in the fields of connective tissue glycoconjugates, immunolocalisation or cell culture would be an advantage. Starting salary in the range £4,333 to £4,910 p.a.

Applications with full curriculum vitae and the names of two referees should be sent, as soon as possible, to Professor D.S. Jackson, Department of Medical Biochemistry, University of Manchester Medical School, Manchester, M13 9PT.

3142(A)

POST-DOCTORAL RESEARCH ASSISANT required in Department of Microbioloy to work on a three-year M.R.C. project on latency of human papovaviruses. Suitable for biochemist or virologist with experience in nucleic acid research. Salary not less than £4,262 plus £502 London Allowance for suitably qualified person. For further information telephone Dr McCance (01-407 7600 ext. 3376). Apply in writing, with full curriculum vitae, to the Secretary, Guy's Hospital Medical School, London Bridge, SE1 9RT, quoting Ref. M.C.5. 3078(A)

UNIVERSITY OF BRISTOL

CHAIR IN PHYSIOLOGY

The University invites applications for appointment to the Chair in Physiology which becomes vacant on the appointment of Professor T.J. Biscoe to the Jodrell Chair of Physiology at University College, London. The appointment will carry with it Headship of the Department for a five year period in the first instance.

A clinical qualification is desirable but not essential. Candidates are invited to submit applications, including the names of three referees, not later than 12th October, 1979, to the Registrar and Secretary of the University, Senate House, Bristol, BS8 1TH, from whom further particulars may be obtained. 3134(A)

LEEDS AREA HEALTH AUTHORITY (TEACHING)
EASTERN DISTRICT

ST JAMES'S UNIVERSITY HOSPITAL

SCIENTIFIC OFFICERS

Applications are invited from graduates with an Honours degree in one of the Biological Sciences for additional posts of Scientific Officer in the Regional Cytogenetic Unit established at this hospital, undertaking investigations required for patients within a wide area in and around Leeds.

Previous experience in cytogenetics is desirable but not essential.

Application forms & job descriptions available from the Personnel Office — General Sector, Gledhow Wing, St James's University Hospital, Beckett Street, Leeds LS9 7TF. Tel. Leeds 33144, ext. 5103. 3117(A)

RESEARCH APPOINTMENT

he European Economic Community is to upport a European programme of esearch on Dutch elm disease. As part of his programme the Forestry Commission s undertaking a wide ranging study of the ariability of the causal fungus *eratocystis ulmi* and requires a Scientific fficer at the Forestry Commission esearch Station at Farnham, Surrey to rovide technical assistance with aboratory studies on isolates of *C. ulmi* rom various parts of Europe and lsewhere.

Conditions: Period appointment for 2 ears with a possible extension to a third ear.

Qualifications: A degree in a biological r botanical subject and preferably xperience or special interest in mycology.

Age: Under 27 years of age on 1 December, 1979.

Salary: Within the range for Scientific fficer (£3,289 to £5,085) depending upon ge and experience.

Closing date for applications: pplication forms and further details may e obtained from Mr A.J. Freeman, ersonnel Branch, Forestry Commission, 31 Corstorphine Road, Edinburgh EH12 AT. Completed application forms should e returned no later than 27th September, 979. 3120(A)

UNIVERSITY OF BRISTOL

Cosmic Ray Research

pplications are invited for three posts of

RESEARCH ASSISTANT

ach of 24 months duration, for work ithin the cosmic ray studies group under rofessor P.H. Fowler, FRS. These posts ill be concerned with experiments to easure the high energy component of the ltra-heavy cosmic rays and the 'anomalous'' component of the low nergy cosmic rays.

Experience with nuclear or spacecraft strumentation, especially the use of drift hambers and microprocessor based data cquisition sytems, would be appropriate.

Salaries will be on the scales for research taff grades 1A (2 posts) £4,333 × £289 and B (1 post) £3,775 × £280. (Scale effective rom 1st October, 1979 and subject to urther review).

Applications, naming two referees and iving details of education and relevant xperience should be made by 20th eptember, 1979 to Dr R.M. Redfern, .H. Wills Physics Laboratory, Tyndall venue, Bristol BS8 1TL. Telephone ristol 24161 ext 110, from whom further nformation can be obtained. 3047(A)

UNIVERSITY OF THE WITWATERSRAND

Johannesburg

DEPARTMENT OF GEOLOGY

Applications are invited for appointment to the following posts:

CHAIR OF MINERAL GEOCHEMISTRY

The successful applicant for the above teaching and research position will be responsible for the co-ordination and advancement of teaching courses in geochemistry in the department, and, in addition to pursuing his/her own research projects, will be encouraged to participate in co-operative research programmes in a wide range of interdisciplinary Earth Science projects, and to collaborate with the substantial group of geochemists in other sections of the University. The Department is well equipped with an experimental geochemical laboratory and XRF, XRD and wet-chemical analytical facilities; a modern ARL-SEMQ electron microprobe has recently been installed, and is operated and maintained by an electron microprobe technical officer.

The starting salary will be determined according to qualifications and experience in the range R15,000 to R18,000 per annum. In addition, the appointment carries a non-pensionable supplementation of R4,000 per annum.

LECTURESHIP IN ENGINEERING GEOLOGY

The principal duties attached to this post include assisting in the teaching of a one-year introductory course in Geology to Engineering students, and participation in more advanced courses in Engineering Geology in the newly-introduced four-year BSc (Eng) degree course in this field.

The starting salary will be determined according to qualifications and experience in the range R8,100 to R13,200 per annum.

The University's policy is not to discriminate in the appointment of staff or the selection of students on the grounds of sex, race, religion or colour. Further particulars relating to this practice and to the above posts are included in an information sheet obtainable from the London Representative, University of the Witwatersrand, Chichester House, 278 High Holborn, London WC1V 7HE, or from the Registrar, University of the Witwatersrand, Jan Smuts Avenue, Johannesburg, 2001 South Africa, with whom applications should be lodged by 28th September, 1979. 3110(A)

RESEARCH & DEVELOPMENT VACANCIES

Vacancies exist in the Research & Development department of a large international company for Materials Scientists and Applied Physicists to work in the areas of Physics, Optics and Electro-optics in a small but expanding Research Team. Candidates must possess a BSc in Physics or Applied Physical Sciences, or a higher qualification, and will probably be new or recent graduates with an aptitude for experimental and practical work.

There is also an opening for an Applied Mathematician or Theoretical Physicist with at least two years' experience in mathematical modeling, using both high and low-level programing techniques. Many of the problems are time constrained and there will be a requirement for the development of real-time algorithms in addition to solutions for complex geometrical problems.

This is an unusual opportunity to join a unique organisation offering excellent conditions of employment with opportunities for overseas travel from time to time. These posts will be based initially in Central London although the R&D department will be moving to premises to the West of London in the future.

Please write with Curriculum Vitae to:

M. H. Boorman,
2, Charterhouse Street,
London, EC1N 6RX.

3139(A)

South London College

formerly Norwood Technical College,
founded 1859
Main Building: Knights Hill SE27 0TX
Tower Bridge Branch: Tooley Street SE1 2JR

Department of Physical Sciences

Research Assistantship in Geology

The College has a Research Assistantship in Geology available from 1 October 1979, initially for a period of one year but extendable to a maximum of three (depending on satisfactory progress) to enable a PhD to be obtained if appropriate, to be based in the Geology section of the Department which is located at Knights Hill.

Applicants should have a good Honours degree in Geology or Earth Sciences.

The successful applicant will have the opportunity to research in volcanology, or in the petrology and mineralogy of volcanic rocks, or in the magmagenesis of volcanic rocks.

Salary Scale: £2,613 × £87(2) – £2,787 (under review) plus London Allowance of £474.

Application forms, returnable within 14 days of the appearance of this advertisement, obtainable from the Clerk to the Governors at the main building. Enclose self-addressed envelope, not smaller than 10" × 7" if a Prospectus and any further particulars are required.

ilea INNER LONDON EDUCATION AUTHORITY 3118(A)

LABORATORY SCIENTIFIC OFFICER required in Department of Microbiology to work on a three-year M.R.C. project on latency of human papovaviruses. Experience in biochemical aspects of virology essential. Salary for qualified Laboraory Scientific Officer not less than £3,261 (under revision) plus £354 London Weighting. Apply in writing, stating age and giving details of qualifications and experience, to the Secretary, Guy's Hospital Medical School, London Bridge, SE1 9RT, quoting Ref. M.C.4. 3077(A)

UNIVERSITY OF OXFORD

in association with
St. Catherine's College

TEMPORARY TEACHING APPOINTMENT

in Inorganic Chemistry or Chemical Crystallography

Applications are invited for the above appointment on the university lecturer scale, £4,232 to £9,000 according to age, for 5 years to cover a temporary secondment from 1 April 1980. The successful candidate may be offered a Teaching Appointment in Inorganic Chemistry by St. Catherine's College for the duration of the university appointment.

Details of the college and university appointments may be obtained from Professor John B. Goodenough, Inorganic Chemistry Laboratory, South Parks Road, Oxford, OX1 3QR, to whom applications (5 copies) should be sent by 1 November, 1979. 3113(A)

X-RAY CRYSTALLOGRAPHER Research Associate to conduct x-ray diffraction studies on liquid crystals in well equipped laboratory. Required PhD or equivalent experience. Salary: $11-15,000 annually, depending on qualifications. Reply to Dr Leonid V Azaroff, Director, Institute of Materials Science, University of Connecticut, Storrs, CT 06268 before October 22, 1979. An Equal Opportunity Employer. XW328(A)

UNIVERSITY OF HONG KONG

CHAIR IN CLINICAL PHARMACOLOGY

Applications are invited for the above-mentioned post in the Department of Medicine. Candidates should have qualifications of M.R.C.P. or equivalent with appropriate experience in patient management and the subject required.

Annaul salary (currently under review) (superannuable) will be within the professiorial range and not less than HK$197,280 (£1 = HK$11.50 approx.).

At current rates, salaries tax will not exceed 15% of gross income. Housing at a rental of 7½% of salary, education allowances, long leave and medical benefits are provided.

Further particulars and application forms may be obtained from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or the Assistant Secretary (Recruitment), University of Hong Kong, Hong Kong.

Closing date for applications is *1 October 1979.* 3098(A)

The Queen's
University of Belfast

RESEARCH ASSISTANT

Palaeoecology Laboratory

Applications are invited from graduates in physical or biological sciences for this Science Research Council financed post tenable for three years. The successful applicant will be required to carry out tree-ring dating and timber sample preparation for which full training will be given. The job demands an exacting standard of accuracy and will involve a high proportion of microscope work. A driving licence would be an advantage.

Commencing salary in the range £3,777 to £4,335.

Curriculum vitae and the names and addresses of two referees should be sent to the Personnel Officer, The Queen's University of Belfast, BT7 1NN, Northern Ireland. Closing date: 21st September, 1979.

3119(A)

MEDICAL RESEARCH COUNCIL

National Institute for Medical Research

IMMUNOLOGIST

A young postdoctoral immunologist is required by the Laboratory for Leprosy and Mycobacterial Research to investigate immunity of mycobacterial infections. It is proposed that the person appointed will study particularly the immunological control of the growth of mycobacteria in tissue culture and in animals. The appointment will be for up to three years and will be in the salary range £4,940 to £6,627 per annum according to age and qualifications, plus £502 p.a. London allowance. Superannuation provision.

Applications, giving details of qualificaions, experience and the names of two professional referees, should be sent to the Director, National Institute for Medical Research, Mill Hill, London NW7 1AA before 31st October 1979, quoting reference LM. 3130(A)

ST. HELIER HOSPITAL CARSHALTON, SURREY

SCIENTIFIC ASSISTANT

DERMATOLOGY DEPARTMENT

With post graduate experience in either Biochemistry or Immunology to work on skin diseases including malignancy.

Apply by 28th September to Dr E.L. Rhodes at above address.

3100(A)

SIMON FRASER UNIVERSITY

Burnaby, British Columbia

DEPARTMENT OF CHEMISTRY

APPOINTMENT IN BIOCHEMISTRY

Applications are invited for a tenure track appointment at the assistant professor rank, preferably with specialization in the area of macro-molecular biochemistry, starting September 1, 1980. The appointment is subject to budgetary approval. The successful applicant will be required to teach graduate and undergraduate courses in biochemistry and to establish a vigorous research program.

The current base of the assistant professor rank is $19,720 per annum.

Applications, including curriculum vitae, a concise statement of proposed research and the names of three referees, should be sent by December 31, 1979 to: Dr. C.H.W. Jones, Chairman, Department of Chemistry, Simon Fraser University, Burnaby, BC, Canada V5A 1S6. W319(A)

UNIVERSITY OF DUBLIN
Trinity College

LECTURER IN PHARMACEUTICS

Applications are invited for the post of Lecturer in Pharmaceutics in the School of Pharmacy, Trinity College, Dublin. Preference will be given to pharmacy graduates with suitable experience in Pharmaceutical Technology or Physical Pharmaceutics.

Salary Scale: £5,078 to £10,038 p.a.

Appointment is likely to be made within the range £5,078 to £6,462 p.a. There is a non-contributory pension scheme.

Application forms and further particulars relating to this post may be obtained from:

The Establishment Officer
West Theatre
Trinity College
Dublin 2

to whom completed applications should be sent *not later than 25th September, 1979.*

3104(A)

FERMENTATION MICROBIOLOGIST

We are a large forest products R&D organisation, and are expanding our efforts on the conversion of wood residues to valuable products. Applicant should have a good honours degree, and preferably a PhD, in one of the biological sciences, together with specialised knowledge of fermentation processes, ideally in the area of biochemical engineering.

Duties will be to grow microorganisms on wood-based materials in laboratory fermenters, including continuous culture equipment. He will work closely with chemists on suitable pretreatments of the wood substrate and will be expected to devise techniques for screening, collecting and optimizing the yield of a variety of end products.

Salary open; fringe benefits generous. For further information please write to: Mr I B Flann, Forintek Canada Corp, 800 Montreal Road, Ottawa, Ontario, K1G 3Z5, Canada. W330(A)

UNIVERSITY COLLEGE LONDON

READERSHIP IN PROBABILITY AND STATISTICS

The Senate invites applications for th above Readership in the Department o Statistics and Computer Science particularly from persons with researc interests in data analysis. Salary at a agreed point on the scale £8,698 to £10,77 (from October 1979) plus £502 Londo Allowance.

Applications (10 copies) must b received not later than 19 October 1979 b the Academic Registrar (N), University o London, Senate House, London WC1E 7HU, from whom further particular should first be obtained. 3096(A)

LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE

(University of London)
Keppel Street, WC1E 7HT.

DEPARTMENT OF ENTOMOLOGY

A MEDICAL LABORATORY SCIENTIFIC OFFICER

is required to assist for up to 18 months in a WHO-funded programme on *in vitr* culture of human filariae using insect cel lines. Candidates must hold an HNC in a relevant discipline.

Applications, in writing, giving ful career details and naming two referees should be sent to Secretary (A1). 3143(A)

THE UNIVERSITY COLLEGE OF WALES ABERYSTWYTH

Department of Agricultural Botany

Applications are invited for the post of Postgraduate Research Assistant tenable from 1 October, 1979 for a period of three years to work on Nuclear DNA variation in higher plant species. Candidates should have a Degree in Biology with training in Genetics or Biochemistry.

Salary from £3,775 per annum on Range IB of the Research and Analogous Scales.

Application forms can be obtained from The Registrar, (Staffing Office), The University College of Wales, Old College, King Street, Aberystwyth, SY23 2AX (Tel. 0970 3177) to whom completed forms hould be returned no later than Monday, 7th September, 1979. 3127(A)

ASTRONOMER ROYAL FOR SCOTLAND REGIUS CHAIR OF ASTRONOMY UNIVERSITY OF EDINBURGH

he Secretary of State for Scotland invites pplications for the office of Astronomer oyal for Scotland and for the post of egius Professor of Astronomy in the University of Edinburgh. The Science esearch Council will participate in the election of the successful applicant, so hat he/she may be appointed by the ouncil as Director of the Royal bservatory, Edinburgh. It is hoped that he person appointed will assume respon-bilities with effect from 1st October, 980.

Further particulars may be obtained om the Secretary, Scottish Education epartment, 8 George Street, Edinburgh H2 2PF to whom 4 copies of applications, cluding the names of three referees, hould be sent not later than Monday, 8th ctober 1979. 3044(A)

POSTDOCTORAL RESEARCH ASSOCIATE

o work on the organisation and anscription of the genes for mammalian ow molecular weight RNAs, or on the unction of these RNA species. Recent hD with background in molecular biology r biochemistry preferred.

Send C.V. and names of three references o: Dr. George L. Eliceiri, Cell Biology ivision, Department of Pathology, St. ouis University School of Medicine, 1402 Grand, St Louis, Missouri 63104, U.S.A. n Equal Opportunity Employer M/F/H. W331(A)

FRESHWATER IOLOGICAL ASSOCIATION

SENIOR ANALYTICAL CHEMIST

acancy at Windermere Laboratory. uties to include: general co-ordination of alytical programme and development of ose liaison with biological staff; routine alysis; development of new methods; ersight of radiochemical laboratory, cluding training of scientific staff in chniques and safety.

Candidates should hold a first degree or evant industrial qualification and have nsiderable practical experience. The nsionable post is graded HSO/SSO and arting salary would be £5,800 or above pprox. 10% additional pay award is due n January 1980).

Further details and application forms ay be obtained from: The Secretary, eshwater Biological Association, The rry House, Ambleside, Cumbria LA22 P. Closing date 14th September 1979. 2979(A)

UNIVERSITY OF NOTTINGHAM

Applications are invited for the post of

RESEARCH ASSISTANT

from recent graduates in a Biological Science to investigate the pharmacology of cultured mouse neurones. This project is supported by MRC and will be under the direction of Professor P.N.R. Usherwood.

Appointment for one year in the first instance (with a possible extension for two years) from October 1979 or as soon as possible thereafter. Salary on Scale £3,883 to £4,382.

Applications, including full curriculm vitae and names of two academic referees to: Professor P.N.R. Usherwood, Zoology Department, Nottingham University, University Park, Nottingham NG7 2RD. 3081(A)

UNIVERSITY OF OXFORD

TECHNICIAN IN ELECTRON MICROSCOPY

A Grade 4 technician is required to work on a three year ARC funded project concerned with the development of techniques for the preparation of biological specimens for high voltage electron microscopy. It is an unusual project and there is plenty of scope for initiative. Salary scale in the range £3,432 to £3,950, increasing in April, 1980, to £3,641 to £4,191.

Applicants should preferably have some EM experience and hold ONC, C&G, or equivalent qualifications, but inexperienced candidates will be considered. Further particulars may be obtained from The Administrator, Department of Agricultural Science, Parks Road, Oxford, OX1 3PF, to whom applications, including full curriculum vitae and names and addresses of two referees, should be sent as soon as possible. 3138(A)

THE UNIVERSITY OF BRITISH COLUMBIA

DEPARTMENT OF CHEMISTRY

ASSISTANT PROFESSORSHIP

Applications are invited from well qualified candidates for a position at the rank of Assistant Professor which will become vacant as the result of the retirement of a Faculty member on June 30, 1979. Persons with research and teaching interests in any area of chemical science are eligible to apply.

Applications consisting of a curriculum vitae, a list of publications and the names and addresses of three referees should be sent to the undersigned before November 30th, 1979. This position is open to both male and female applicants.

Professor Charles A. McDowell, Department of Chemistry, University of British Columbia, 2075 Wesbrook Mall, Vancouver, British Columbia, Canada, V6T 1W5. W337(A)

THE UNIVERSITY OF LEEDS

Department of Plant Sciences

Applications are invited for a post of

DEMONSTRATOR

in the above Department for a fixed period of one year from 1 October 1979. Applicants should have a good honours degree in a Biological Science.

Salary on the IB scale for Other Related Staff: £3,775 to £6,355 (under review), according to age, qualifications and experience.

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference number 53/14/D. Applications should be submitted as soon as possible and not later than 21 September, 1979. 3125(A)

News Editor

A senior vacancy exists for News Editor of *Nature*, the international and interdisciplinary journal for scientists and science policy-makers. This vacancy has occurred with the move of the present News Editor to the new post of *Nature*'s Chief European Correspondent.

The News Editor will have responsibility under the Editor for about twelve news pages in each week's issue covering policies, events and personalities of concern to, or influenced by, the international scientific community.

He/she will have the full time services of an American and a European staff correspondent as well as help with lay-out and sub-editing.

The successful candidate will have several years experience as a journalist, a strong interest in international affairs and an enthusiasm for planning, co-ordinating and commissioning. Science background not essential but familiarity with the demands of professional/academic readership an advantage.

Write as soon as possible to — The Editor, *Nature*, Macmillan Journals Limited, 4 Little Essex Street, London WC2R 3LF, enclosing a curriculum vitae.

3048(A)

M MACMILLAN

ASSISTANT PROFESSOR OF MICROBIOLOGY. The Department of Microbiology and Immunology, Washington University School of Medicine invites applications from highly qualified individuals for an appointment as Assistant Professor. Applicants should have PhD or MD degrees, 2-5 years of post-doctoral research experience and a commitment to medical and graduate student teaching. Teaching competence should be in pathogenic microbiology; research interests should be in microbiology. Send curriculum vitae, brief description of research plans and names of three referees to Dr Joseph M Davie, Department of Microbiology and Immunology, Washington University School of Medicine, St Louis MO 63110 by November 1, 1979. Equal Opportunity and Affirmative Action Employer M/F/H. W339(A)

THE LONDON HOSPITAL MEDICAL COLLEGE (University of London)

RESEARCH TECHNICIAN

Required in the Medical Unit to assist in M.R.C. supported project on hypertension.

The post is for a maximum of two and half years. Previous laboratory experience is expected and experience in steroid immunoassay and/or catecholamine assay would be an advantage. Candidates should have HNC, MIBol, B.Sc. or equivalent.

Initial salary £3,261 + £354 London Allowance per annum (under review).

Enquiries to Dr H.L.J. Makin (01-247-0644, Ext.71). Applications to the Secretary, The London Hospital Medical College, Turner Street, London E1 2AD, quoting ref. RTMU/9/79. 3084(A)

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY

DEPARTMENT OF BIOCHEMISTRY

POST-DOCTORAL RESEARCH ASSISTANT IN MOLECULAR BIOLOGY

Applications are invited for the above position to work on the genome organisation and expression of a plant virus with a circular single-stranded DNA genome. The investigation is a prelude to utilisation of the virus DNA as a transforming vehicle for the genetic modification of plant cells. Experience in some aspect of nucleic acid or protein biochemistry would be desirable. The post, which is supported by the science research council, is available for a maximum of three years, with a starting salary on research grade 1A within the range £4,260 to £4,805 p.a. To be reviewed 1/10/79., plus London allowance of £502 p.a.

Applications including curriculum vitae and names and addresses of two referees should be sent to Dr K.W. Buck, Department of Biochemistry, Imperial College of Science and Technology, London SW7 2AZ. 3027(A)

APPOINTMENTS VACANT

ODENSE UNIVERSITY
Denmark

Applications are invited for a position as lecturer at the Department of Molecular Biologi.

The new lecturer is supposed to participate in the plasmid group at the department. The research program of this group is concentrated on the replication (particularly control of replication) and biological importance of plasmids. Consequently, we are particularly interested in applicants with experience in molecular, biochemical, genetic, and physiological studies of bacteria.

The new member of the department is supposed to teach molecular biology and microbiology at all levels.

Enquires to Dr Kurt Nordström, Department of Molecular Biology, University of Odense, Campusvej 55, DK-5230 Odense M, Denmark. Telephone (09) 158600, extn. 2375. The new lecturer is expected to be able to teach in Danish after a few years. A professional selection committee will discuss the applications and their recommendation will be sent out to all applicants in its complete form.

Conditions of employment as provided by an agreement between the Ministry of Finance and the trade union of the applicant preferred.

Application in 3 copies, marked "position nr. 936" including a curriculum vitae, information of teaching experience, reprints of recent publications, and the names of referees should be sent (in triplicate) to: Odense universitet, journalkontore, Campusvej 55, DK-5230 Odense M, not later than Oktober the 4th, 1979. W346(A)

BIOCHEMIST

BIOCHEMICAL PHARMACOLOGIST

PROTEIN CHEMIST

To work on purification of enzymes that metabolize peptides and on enzyme inhibitors, as postdoctoral fellow or as visiting scientist, immediately.

Please contact Norene Ross, Secretary, Department of Pharmacology, University of Texas Health Science Center, Dallas, Texas 75235. W347(A)

KING'S COLLEGE LONDON
DEPARTMENT OF PHYSICS
LABORATORY TECHNICIAN GRADE 5

Responsibilities include developing, constructing and modifying apparatus for teaching requirements and student projects etc; maintenance, servicing and repairing of equipment and the preparation of graphs, blueprints and engineering drawings. The post also involves the training of juniors and some supervision of other technical staff. Applicants should have suitable qualifications and experience. Salary on scale £4,224 p.a. inclusive rising to £4,844 p.a. (under review). 5 weeks annual holiday. Contributory pension scheme. Interest-free loans for annual rail season tickets.

Apply in writing with full details to The Head Clerk (Ref. 217835/N), King's College London, Strand, WC2R 2LS.

3105(A)

FELLOWSHIPS and STUDENTSHIPS

UNIVERSITY OF ESSEX
DEPARTMENT OF PHYSICS
SRC POSTDOCTORAL RESEARCH FELLOWSHIP
Theory of Surface Excitations on Solids and Liquids

Applications are invited for this three-year fellowship to commence in January 1980 or as soon as possible thereafter. The initial salary will be on one of the first three points of the 1A scale (currently £4,333 to £7,521).

The successful candidate will work with M.G. Cottam, R. Loudon and D.R. Tilley on the theories of various kinds of surface excitation, including magnetic, electronic and vibrational surface waves on solids, and surface ripples on liquids. The main aim of the work is the prediction and interpretation of the experimental light-scattering spectra of surface excitations. The techniques employed in the calculations include standard solid-state theory of spin waves, lattice vibrations and excitons, linear-response theory, light-scattering theory, and fluid mechanics; applicants should have prior research experience in at least one of these areas.

Applications by letter, including a curriculum vitae and the names and addresses of two referees, should be sent to Dr M.G. Cottam, Department of Physics, University of Essex, Colchester CO4 3SQ, from whom further particulars may be obtained, by 20 October 1979. 3088(E)

THE UNIVERSITY OF NEWCASTLE UPON TYNE
DEPARTMENT OF ORGANIC CHEMISTRY

Applications are invited for a

RESEARCH STUDENTSHIP

from persons, who have a good honours degree in Chemistry, to work on the synthesis of novel ß-lactam antibiotics. The value of the award will be at current SRC rates and the student will be expected to register for a higher degree.

Applications, together with the names of two referees, should be sent as soon as possible to Dr R.J. Stoodley, Department of Organic Chemistry, The University, Newcastle upon Tyne, NE1 7RU.

3080(F)

UNIVERSITY OF ST ANDREWS
Department of Biochemistry
ARC Postdoctoral Research Fellowship

Applications are invited for a postdoctoral Research Fellowship to investigate the role of intracellular proteinases in the regulation of enzyme levels in barley under the direction of Dr. J.L. Wray. The project will involve the identification and purification of proteinases and characterisation of their role in the degradation of nitrate reductase and nitrite reductase. Preference will be given to applicants with experience in enzyme purification and/or immunochemical techniques.

Starting salary up to Point 3 (£4,910 on Grade 1A of the national salary scales for research staff (at present under review), plus USS. The appointment will be for three years.

Applications (two copies preferably in typescript) including a curriculum vitae and the names of two referees should be sent by 1st October 1979 to the Establishments Officer, The University, College Gate, St. Andrews, Fife KY16 9AJ from whom further particulars may be obtained. 3116(E)

UCG Coláiste na hOllscoile Gaillimh

Department of Zoology
POST-DOCTORAL FELLOWSHIP QUANTITATIVE GENETICS

Applications are invited from suitably qualified candidates for appointment to the above position. The appointee will join a small team being assembled at Galway to study the application of genetics to the improvement of cultivated fish and shellfish. The Department runs its own shellfish hatchery at Carna, approximately 50 miles from Galway. The appointee will work in conjunction with the staff of the hatchery but he/she will be based at Galway.

Candidates should have a good Honours Degree in an appropriate subject (e.g. zoology, genetics) and PhD Degree or equivalent research experience.

The position offers scope for development of the candidate's own research interest within the overall programme of the genetics team. Previous training and experience in quantitative genetics highly desirable.

Letters of application should be **forwarded before 5th October 1979** to the undersigned from whom further details are available:

Prof. N.P. Wilkins, Ref. Quant. Genet.,
National University of Ireland,
University College,
Galway, Ireland.

Recruitment will commence on or before 2nd November, 1979.

3115(E)

EARTH AND PLANETARY SCIENCES

The Lamont-Doherty Geological Observatory of Columbia University invites scientists interested in any field of the earth and planetary sciences to apply for the following fellowships: two postdoctoral fellowships, each awarded for a period of one year (extendable to two years in special instances) beginning in September 1980 with a stipend of $18,500 per annum. Completed applications are to be returned by January 15, 1980.

Application forms may be obtained by writing to the Director, Lamont-Doherty Geological Observatory, Palisades, New York 10964. Award announcements will be made February 28, 1980 or shortly thereafter. W344(E)

SCIENCE RESEARCH COUNCIL
FELLOWSHIPS
and
OVERSEAS (NATO) FELLOWSHIPS AND STUDENTSHIPS

Applications for SRC Fellowships and Overseas (NATO) Fellowships and Studentships commencing in 1980/81 should be made to the Science Research Council, PO Box 18, Swindon SN2 1ET.

For application forms telephone:

Swindon (0793) 26222, Ext. 2172

Closing dates for applications:

Senior Fellowships	: November 30, 1979
Advanced Fellowships	: September 30, 1979
Postdoctoral Fellowships	: January 31, 1980
Industrial Visiting Fellowships	: Apply at any time
Overseas (NATO) Fellowships	
In Europe	: January 12, 1980 and May 10, 1980
Outside Europe	: January 31, 1980
Overseas (NATO) Studentships	: January 31, 1980

Senior Fellowships candidates, who wish to learn results a year in advance, should also apply by November 30, 1979 for awards commencing in 1981/82. 3099(E)

FELLOWSHIPS continued

THE UNIVERSITY OF BRITISH COLUMBIA
DEPARTMENT OF CHEMISTRY
POSTDOCTORAL RESEARCH POSITIONS

pplications are invited from well ualified candidates for one or two year ppointments as Postdoctoral Research ellows in the following fields: tmospheric photochemistry; Bioinrganic chemistry; Catalysis studies, iffusion and phase separation on rfaces; Catalysis using transition metal omplexes; Marine natural products; evelopment of New Synthetic Methods in rganic Chemistry; Electron impact and hotoelectron Spectroscopy including uger and ESCA studies; Electron paraagnetic, ENDOR, NOR, Mossbauer, icrowave, electronic and LEED ectroscopies; Flash photolysis and inetic Spectroscopy; Fluorocarbon hemistry; Homogeneous and Heteroeneous Catalysis; Immunochemistry or olysaccharides; Inorganic photochemtry and photophysics; Kinetic theory of ases applied to aeronomy and space ience; Lipid synthesis for NMR study of iomembranes; Mechanism of the tercalation of layered Structures; uonium Chemistry at TRIUMF: NMR d laser Raman spectroscopy of nuclear in labelled nucleic acids; Nonlinear ptical effects in organic solids at low mperature; Nuclear magnetic resonance udies of molecular motion; Organic Solid tate Reactions; Organometallic Phoshazene and Thiazyl Chemistry; Oxidion, use of coenzyme models, general id catalysis; Potential antiviral and titumor compound synthesis; Quantum emistry and Molecular Physics; adiation chemistry and solvated electron udies; Synthesis and study of organic etals; Theory of molecular relaxation and ansport properties; X-ray diffraction udies.

The minimum stipend for the first year ill be $11,880. A substantial number of eaching Postdoctoral Fellowships, with a inimum stipend of $14,000, are open to udents who have recently graduated and ave shown superior ability in research and holarship.

Send curriculum vitae, transcripts, and tters from three referees to the undergned, from whom further particulars may obtained. Professor C.A. McDowell, epartment of Chemistry, University of ritish Columbia, Vancouver, BC, V6T W5, Canada. W338(E)

EARTH AND PLANETARY SCIENCES

he Lamont-Doherty Geological bservatory of Columbia University vites scientists interested in any field of e earth and planetary sciences to apply r the following fellowships: two ostdoctoral fellowships, each awarded for period of one year (extendable to two ars in special instances) beginning in eptember 1980 with a stipend of $18,500 r annum. Completed applications are be returned by January 15, 1980.

Application forms may be obtained by riting to the Director, Lamont-Doherty eological Observatory, Palisades, New ork 10964, Award announcements will be ade February 28, 1980 or shortly ereafter. W336(E)

SANDOZ RESEARCH INSTITUTE
Vienna, Austria

is one of three international research centres of the Swiss based pharmaceutical company SANDOZ.

Currently, we offer a

POSTDOCTORAL FELLOWSHIP

in our Department of IMMUNOLOGY for research on mechanisms of natural and acquired resistance to herpes- and influenza-virus infections in mice and man. The candidate should hold a PhD or an equivalent degree. Background in cellular immunology, immunogenetics and/or viral immunology preferred.

The successful candidate will find excellent working conditions in well equipped laboratories. The appointment will be for an initial 2-year period. We offer an attractive salary. Travel expenses from and to the country of origin will be paid.

Please send application with curriculum vitae, two references and list of publications to

SANDOZ

Dr. N. Zacherl, Personnel Manager,
SANDOZ Forschungsinstitut,
Brunner Straße 59,
A-1235 Vienna, Austria

W333(E)

TO PLACE YOUR ADVERTISEMENT IN THESE PAGES

Phone : 01-831-6901

INSTITUTE OF CANCER RESEARCH: A three year Fellowship is offered in the Division of Molecular Biology for a Post-Doctoral Scientist to work on the isolation and characterisation of non-histone chromosomal proteins from plants and lower eukaryotes. Salary in range £4,261 to £4,805 pa plus London Allowance of £502 pa. Applications in duplicate with the names of two referees to the Secretary, Institute of Cancer Research, 34 Sumner Place, London, SW7 3NU, quoting ref. 300/G/13. 3122(E)

NUFFIELD FOUNDATION
ONE-YEAR SCIENCE RESEARCH FELLOWSHIPS

The Nuffield Foundation is offering to University Lecturers in Science (but not Mathematics) Departments a number of one-year research fellowships to be awarded competitively at the end of 1979 and to be held during the academic year 1980-81. The objective is to enable younger university lecturers to be freed from teaching and administrative duties for one academic year in the first instance; preference will therefore be given to applicants under the age of 40. Awards will cover the cost of replacement teaching and will include an allowance for research expenses.

The closing date is 15 October 1979 and further particulars may be had from the Director, Nuffield Foundation, Nuffield Lodge, Regent's Park, London NW1 4RS 3133(E)

STRANGEWAYS RESEARCH LABORATORY
Cambridge
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available for work on the causation of lung cancer by environmental and industrial metal particulates and the possible interaction of these with systemic agents. The project is based in Cambridge and involves collaboration with the Industrial Toxicology Unit of the Dept of Safety and Hygiene, University of Aston. Funding is provided by a 3 year grant from the Health and Safety Executive starting as soon as practical. MRC conditions of service, starting salary in the range £5,048 to £5,850 depending on age and qualifications. Applications (2 copies) from persons with good previous experience of the techniques of experimental pathology should be forwarded to Administrative Secretary, Strangeways Research Laboratory, Worts Causeway, Cambridge CB1 4RN, together with full c.v. and the names of two referees. 3124(E)

THE ROYAL SOCIETY
ROYAL SOCIETY METEOROLOGICAL OFFICE RESEARCH FELLOWSHIP IN DYNAMICAL METEOROLOGY

Applications are invited by the Council of the Royal Society for a

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tenable in the department of atmospheric physics, University of Oxford. Candidates should supply the usual personal details and the names of two referees, together with a full description of the research proposed.

The appointment, which will be subject to the Society's general regulations governing research appointments, will be tenable for five years from a date to be mutually agreed in the academic year starting on 1 October 1979. The commencing salary which will depend on age and experience, will be within the scale £4,863 to £8,019 per annum, with appropriate superannuation benefits.

Applications should be made on forms to be obtained from The Executive Secretary, The Royal Society, 6 Carlton House Terrace, London, SW1Y 5AG, and should be received not later than 30 September 1979. 3083(E)

FELLOWSHIPS continued

ESO FELLOWSHIPS 1980-1981

The European Southern Observatory (ESO) intends to award up to six fellowships tenable in the ESO Scientific-Technical Center which from August 1980 will be located in Garching near München.
The main goals of the Center are as follows:
— to carry out a program of development of auxiliary instrumentation for the La Silla telescopes;
— to make studies in observational and theoretical astrophysics so that the observing facilities may be used in an optimal way;
— to provide data reduction facilities to users of the ESO instruments;
— to provide photographic facilities for atlases of the southern sky;
— to foster co-operation in astronomy and astrophysics in Europe.
Most of the scientists in the Center come from the Member States of ESO, but several are from other countries. At present, the Member States of ESO are: Belgium, Denmark, the Federal Republic of Germany, France, the Netherlands and Sweden. In addition to regular staff members, the Center comprises visiting scientists and post-doctoral fellows.
ESO facilities include the La Silla Observatory in Chile with its six telescopes in the range of 0.9 to 3.6m, as well as a 1m Schmidt and some smaller instruments. In Garching, extensive measuring, image processing and computing facilities will be available.
Applicants normally should have a doctorate. The basic monthly salary will be not less than DM2,991. The fellowships are granted for one year, beginning October 1, 1980, with reasonable possibilities for renewal for a second year. Applications should be submitted to ESO not later than December 31, 1979. Applicants will be notified by the end of February 1980. The ESO Fellowship Application form should be used and be accompanied by a list of publications. In addition, three letters of recommendation should be obtained from persons familiar with the scientific work of the applicant. These letters should reach ESO not later than December 31, 1979.
Enquiries, requests for application forms and applications should be addressed to:

European Southern Observatory
Fellowship Program
Schleissheimer Strasse 17
D-8046 GARCHING b. München
Federal Republic of Germany

W332(E)

ASSISTANTSHIPS

UNIVERSITY OF LEICESTER

Department of Biochemistry

POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for a postdoctoral research assistantship supported by the MRC to work with Dr D Critchley on cell surface changes and malignancy. The project is directed toward investigating the possible relevance of loss of complex glycolipids to other aspects of the malignant phenotype, including loss of fibronectin. Experience in cell culture and membrane biochemistry is desirable but not essential.

The appointment will commence as soon as possible; the post is most suited to candidates with at least 1 or 2 years postdoctoral experience, although recently qualified candidates will be considered. The salary will commence in the range of £4,333 to £5,199 depending on the candidate's age and experience.

Applications including curriculum vitae and the names of two referees should be sent to Dr D Critchley, Department of Biochemistry, University of Leicester, Leicester LE1 7RH. 3126(P)

UNIVERSITY OF DURHAM

DEPARTMENT OF ZOOLOGY

POST-DOCTORAL BIOCHEMIST/PHYSIOLOGIST

required for a three year Senior Research Assistantship tenable as soon as possible, to investigate the control of lipogenesis and membrane biogenesis during hormone induced cell differentiation in avian liver. Research experience in biochemistry or a related field is essential and experience of cell culture/enzymology/immunochemistry would be an advantage. Initial salary in the range £4,333 to £4,910 per annum within Range 1A plus superannuation.

Applications (3 copies) naming three referees should be sent by 28 September 1979, to the Registrar and Secretary, Science Laboratories, South Road, Durham, DH1 3LE, from whom further particulars may be obtained. 3064(P)

ANNOUNCEMENTS

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COURSES

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Further details and application forms from Dr M L Hooper, Institute of Genetics, Church Street, Glasgow G11 5JS, Scotland. *Closing date for applictions: 30 November, 1979.*

3132(D)

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CONFERENCES

THE NEW YORK ACADEMY OF SCIENCES

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Barbizon-Plaza Hotel,
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Conference Director, The New Yo Academy of Sciences, 2 East 63rd Stree New York, NY 10021. W342(C)

GRANTS and SCHOLARSHIPS

THE ROYAL SOCIETY

SCIENTIFIC PUBLICATIONS GRANT

Applications for grants from the seco allotment of the Scientific Publicatio Grant for 1979 should be made not lat than 22 October 1979 on forms obtaina from the Executive Secretary, The Roy Society, 6 Carlton House Terrac London, SW1Y 5AG. The grant is for t assistance of scientific publications in t United Kingdom, particularly, but n exclusively, those of scientific societies a institutions.

Applications are receivable only fro societies and institutions in the Unit Kingdom and individuals of Briti nationality. 3079(H)

LECTURES

LEUKAEMIA RESEARCH FUND

1979 Guest Lecture will be delivered on Tuesday 23rd October, 1979 at 5.30 pm. at the Institute of Child Health, Guilford Street, London WC1 by
Professor Emil J. Freireich, MD
of the Texas Medical Center, Houston, U.S.A.
subject: **"The Criteria for Curability for Adult Acute Leukaemia"**
Admission is free by ticket from: **Leukaemia Research Fund, 43 Great Ormond Street, London WC1N 3JJ.** tel: 01-405 0101.

3131(K)

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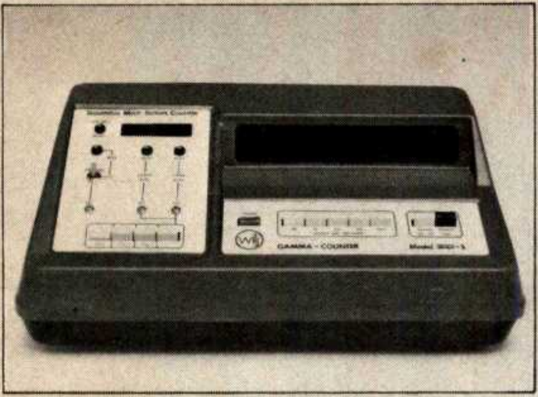

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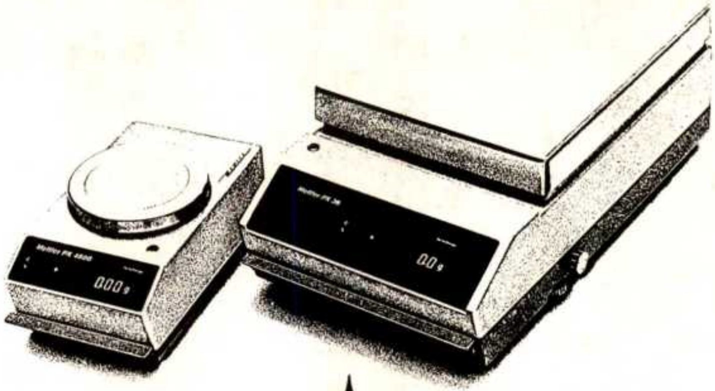

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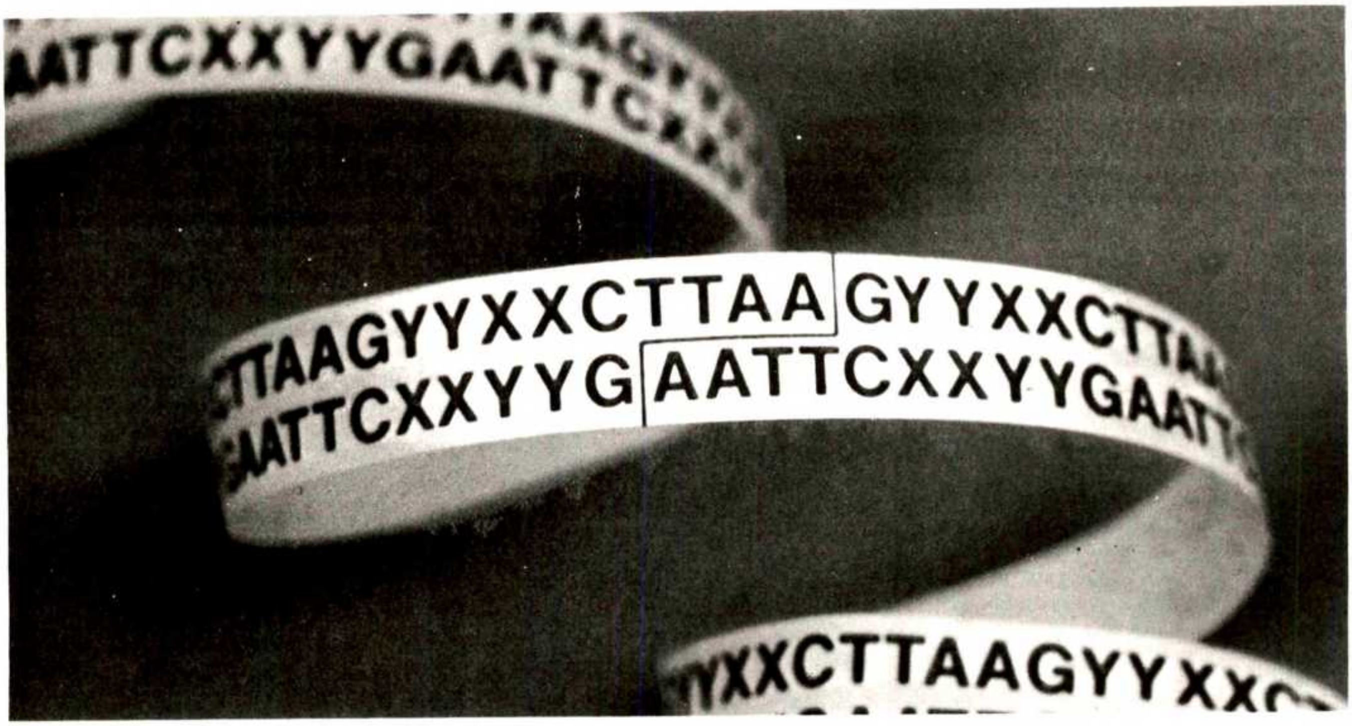

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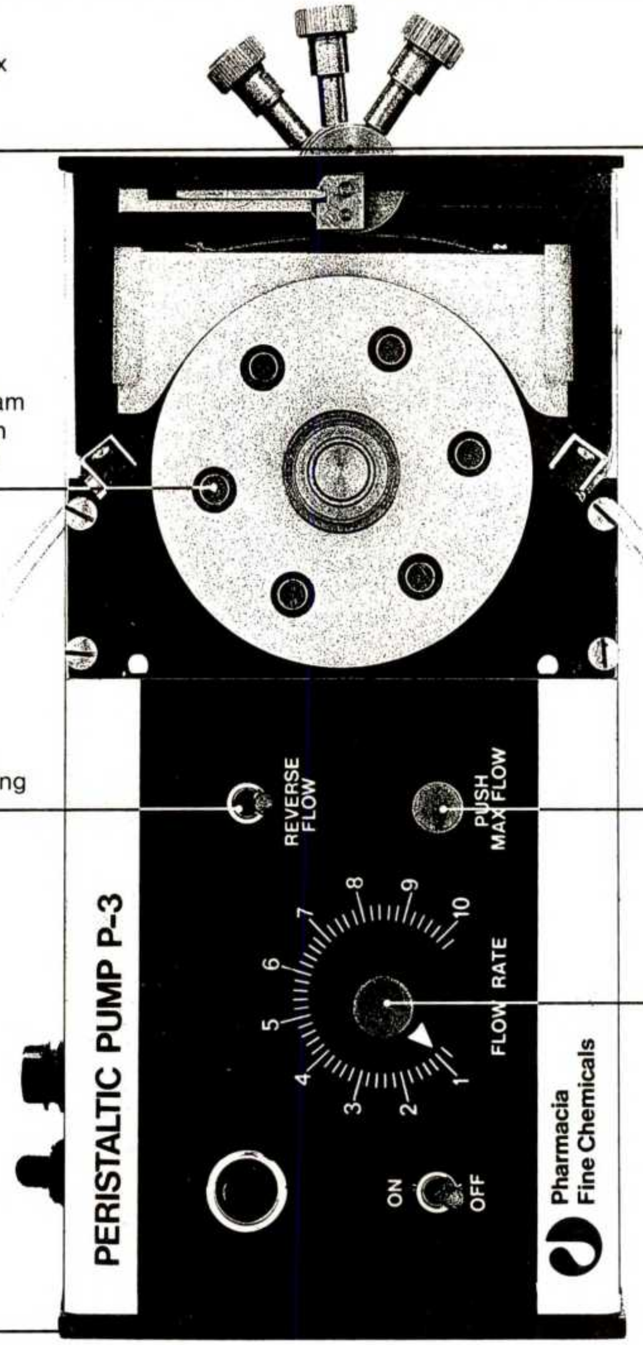

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nature

13 September 1979

Another sort of braindrain

The steady drift of young scientists away from many rural and even urban areas in the UK must be stopped.

One of the easier things for the delegates to UNCSTD, the United Nations Conference on Science and Technology, to decide upon in Vienna recently was that the flow of qualified personnel away from developing countries is one of the most serious obstacles to development. It was equally easy to enjoin developing countries to create the appropriate work environment, and the developed countries to go steady on their recruiting from the Third World. Whether these fine words make an iota of difference to the brain drain is, of course, another matter. And, apparently for political reasons, one type of brain drain — from one developing country to another — was discreetly not mentioned in the Vienna documents, although it is growing in size and could potentially do considerable damage by the syphoning off from one country to another of people with skills of particular relevance to development. Not all the brain drain problems exist in the Third World, however. First World countries can suffer from less publicised but equally disturbing movements of qualified people, and inadequate attention is paid to this problem.

In Britain, as in many other countries, every young person with at least a passing interest in science will certainly be able to study the rudiments of physics, chemistry and biology. In every school with any pretensions to academic quality at all, those who wish to pursue this interest in more detail will be able to concentrate on the sciences, which may additionally include subjects such as geology and psychology, up to the level of university or polytechnic entrance. At this stage, however, for reasons of convenience, the process of concentration starts, and the next three years have to be spent in one of the hundred or so cities in which facilities for higher education exist.

What happens upon graduation? Most but not all of those with scientific qualifications will gravitate towards the academic and teaching world, towards the civil service or towards science-based industry. As far as school-teachers are concerned, opportunities exist, of course, anywhere within the UK. But this is far from true for most other forms of employment. The curious reader might take a list of scientific jobs available outside school-teaching and plot their location on a map. The chances are quite strong that a circle of radius 100 km centred on London will encompass at least 60 per cent of all the vacancies. The chances are equally strong that the number of openings north of the Trent will be less than 20 per cent of the total available.

Scientific jobs in Wales, Scotland and Northern Ireland are particularly rare, as are those in relatively rural parts of the country. But a matter of considerable interest is that jobs in many large cities which achieved their prosperity through industry in the nineteenth century are also relatively uncommon. South-East England is by far the most fertile field for scientific employment in the UK, as it is for many other types of white-collar employment.

Does this matter? In some ways it is a good thing if it ensures a critical mass of scientific expertise capable of sustaining the UK's position in the face of international competition. But whilst there are benefits to be derived from concentration, there are also serious disadvantages. If bright young scientists from large areas of the UK cannot find satisfactory employment in their home area but have to migrate to better-endowed areas, there comes a time when the school-teachers who have been diligently educating these students only to see them all disappear begin to lose heart, and science is listlessly or even badly taught. There comes a time when the scientific awareness of whole regions of the country declines because there are inadequate numbers of scientists around to sustain it. There eventually comes a time when good potential scientists are lost in large numbers.

Centres of excellence are undoubtedly desirable in some academic disciplines, and it would be folly to disperse, say, particle physicists to all corners of the country. But much science-based industry, and many civil-service establishments do not really need to be within close reach of London to be effective; their presence in regions at present less highly populated with scientists might even be of more value than being within an hour or two of the capital city.

There is no obvious time to grasp this problem; the brain drain is slow and makes few headlines. But unless there is careful study of the problem soon, followed by corrective action, the pace of the migration will continue to grow to the detriment of large areas of the country. □

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US expected to exempt most recombinant DNA experiments from federal regulation

Eighty-five per cent of US recombinant DNA experiments will be exempted from controls, if the recommendations of a national committee are accepted. **David Dickson** reports

A large proportion of current experiments using recombinant DNA techniques is expected to become exempt from federal regulations covering such research, following a recommendation which a national advisory committee agreed last week to make to the director of the National Institutes of Health.

In a split decision, the NIH's recombinant DNA Advisory Committee agreed that all experiments using the 'disabled' K12 strain of the bacterium *Escherichia coli* be exempted from the guidelines, except for those which are known to present a particular type of hazard.

The decision has been taken after pressure from scientists, many of whom have argued that the results of recent research fail to support earlier speculations over the hazards of recombinant DNA research. Critics, however, still argue that such a decision is premature, and that there is inadequate knowledge of the risks involved to justify taking such a significant step at this stage.

In its recommendation, the RAC suggests that the experiments exempted from the guidelines should still be carried out under P1 containment conditions, the lowest of four levels of physical containment established in the guidelines which were first published by the NIH in 1976. It also recommends that the experiments should be notified to local institutional biohazard committees, which all institutions carrying out recombinant DNA research on federal funds are required to set up.

However under the committee's recommendations, which are expected to be accepted with little alteration by Dr Donald Fredrickson, director of NIH, there will be no need to obtain prior approval for the experiments from the local committee. Nor will it be necessary to inform the NIH's own Office of Recombinant DNA Activities (ORDA), as all research workers are required to do at present.

Members of the advisory committee voted by ten votes to four, with one abstention, in favour of the exemptions. In general, the scientific members of the committee, many of whom are actively involved in recombinant DNA research or related fields, voted in favour of the exemptions — while those appointed as 'public interest' representatives on the committee voted against them.

During the debate on the proposal, it was said that about 85% of current recombinant DNA experiments were conducted using the *E. coli* K12 strain. The proposed exemptions would not apply to experiments currently prohibited under the guidelines, such as those involving the transfer of genetic material from known toxins, or to experiments involving more than 10 litres of culture — for which special permission is required.

Some members of the committee argued that no decision should taken before the committee had had time to examine the implications of the risk assessment scheme which has been developed by Dr Sidney Brenner of the Medical Research Council laboratory in Cambridge, England. This approach is now being used as a basis for decision-making by Britain's Genetic Manipulation Advisory Group. However other committee members argued that Dr Brenner's concerns were adequately covered by the NIH guidelines. And it was also decided that it was not necessary to wait until Dr Brenner could appear personally before the committee before recommending exemption for the *E. coli* K12 experiments.

During the committee's two-day meeting in Bethesda, approval was given to a set of procedures which have been drawn up by the director of NIH under which private companies can voluntarily register their experiments with ORDA.

Dr Fredrickson told the committee that he was confident that commercially sensitive information could be adequately protected, a factor that had previously made companies reluctant to divulge details of their proposed experiments. He also said that before leaving the Department of Health, Education and Welfare, ex-secretary Mr Joseph Califano had indicated that he was opposed to new legislation covering the private sector.

In a subsequent closed session, the committee gave its approval to large-scale experiments involving more than ten litres of culture submitted by the pharmaceutical company Eli Lilly and the west coast research company Genentech. The two companies are currently collaborating on efforts to produce sufficient quantities of human insulin to begin clinical trials.

The committee also agreed on a set of procedures, based on proposals of a small working group, on how it should conduct its business. Against the advice of the NIH's legal counsel, Mr Richard Riseberg, the committee decided that members having a 'personal stake' in the outcome of a particular line of research should not necessarily be excluded from discussions realted to that research.

Mr Riseberg informed the committee that it might be contravening federal law if, as an advisory committee, members took part in such discussions. Some committee members, however, pointed to the difficulty of determining what 'personal stake' meant in the context of scientific research — and the committee decided to strike out the provision. ☐

US to harmonise remote sensing

The United States has agreed to initiate efforts to "harmonise and rationalise" international activities in the field of remote sensing — the mapping of natural resources from satellites. Such efforts should, according to the US, have two main goals: ensuring the compatibility between the remote sensing systems now being developed by different industrialised countries, and stimulating the full use of remote sensing data by the developing countries.

The decision to take this initiative was announced during the first week of the United Nations Conference on Science and Technology for Development held in Vienna last month, by Father Theodore Hesburgh, head of the US delegation to UNCSTD. Addressing the plenary session of the conference, Father Hesburgh said that the US proposed bringing together the operators of remote sensing satellites, as well as the users, to develop an international system.

"We believe that satellites should be operated so that all can have easy access to the data and so that information can be collected without unnecessary duplication and for maximum mutual benefit", Father Hesburgh said.

"The objective is to ensure developing countries improve their access to information for the use and management of forests, rangelands, water supplies, soil preservation, and the indentifiction of new mineral and water resources."

The Administration has been under pressure from Congress for some time to decide what steps to take following the experience gained with the series of Landsat experimental satellites. Landsat A was launched in 1972, and operated successfully for five years, and its successor Landsat B was launched in 1976. The last of four satellites, Landsat D, is due to be launched in 1981.

Despite the exceptionally high quality of the Landsat images, analysis of them had had mixed success. In some areas, such as the survey of water or mineral resources,

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the satellites have produced a large yield of high quality information. In others, initial goals have proved to be over-optimistic. In particular, it has proved far more difficult than predicted to use satellites for assessing agricultural yields — for example because of problems in distinguishing between different types of crops — and more research is now being done in this area.

Enough information has been gained so far, however, to convince the US that remote sensing can provide useful information to developing countries in fields from water resource engineering (such as building dams or irrigation systems) to mineral prospecting. The US Agency for International Development (AID) has already carried out research projects and training in more than 30 countries, demonstrating how Landsat data can be used by local planners and policy-makers.

Speaking in Vienna last week after Father Hesburgh's address, Mr Tom Pickering, director of the State Department's Bureau of Oceans and the Environment, said that in announcing its new initiative, the US had two main goals, both of which were intended to held developing countries make better use of remote sensing data.

The first goal is to bring together the various countries now planning their own satellite systems to ensure the maximum possible complementarity and compatibility. The aim, for example, would be to ensure not only the minimum of overlap and duplication, but also that wherever possible a developing country receiving station could use the same equipment for receiving data from different satellites.

The second goal is to bring together, possibly on a regional basis, those who intended to use satellite data to obtain information about their own resources, and how these might best be managed. "Our hope is that through discussion with users we can define together the sort of data that can be most effective in answering their needs, and in discovering about their own territories the type of things that they would like to discover," Mr Pickering said.

The Administration's decision was welcomed by Senator Adlai Stevenson Jr, chairman of the Senate's Science and Space Subcommittee, who has long been a critic of the current Administration's lack of plans for an operational Earth-monitoring satellite system, advocating this as one of the most important areas in which US technology can help developing countries.

Describing the wide potential applications of remote sensing, Mr Stevenson said that the US "was concerned that these technologies will follow many national courses and that, if some cooperative steps are not taken soon, it may be too late to at least develop complementary technologies. By complementary we mean not only the physical systems, but also in their operation, so that satellites can be used in a way that avoid redundancies and maximises knowledge of the resources of the world."

Backing up the proposal made in Father Hesburgh's speech, AID mounted an exhibition in Vienna demonstrating the type of data that can be derived from Landsat and other satellites, and the way in which it might be used. The exhibition was to have been entitled "remote sensing: an appropriate technology for economic development" — until advocates in AID of a less capital-intensive approach to Third World needs objected, and the word "appropriate" was dropped from the title.

David Dickson

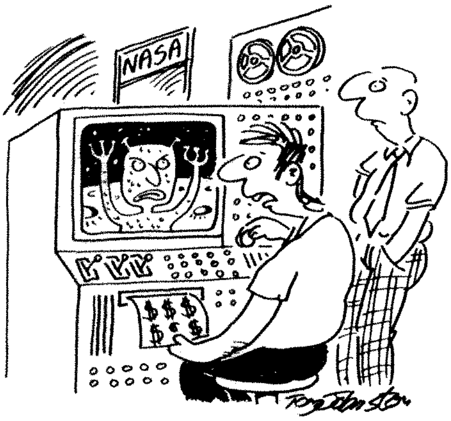
Interference with data from Titan

Vital data on the surface temperature of Saturn's giant moon Titan may have been lost as the result of radio interference during the transmission of the data from the Pioneer 11 spacecraft to the National Aeronautics and Space Administration's Ames Research Center in California last week. In place NASA has a series of dollar signs, printed by the computer receiving the data as an indication that it was unintelligible.

At first it was thought that all the relevant data, which came from readings made by an infrared radiometer aboard the spacecraft and would provide important clues about the planet's atmosphere, had been lost. However subsequent investigation showed that the data had been received — but in a highly garbled form. NASA scientists are now trying to unscramble what they can; they are confident that some of the information can be retrieved, but remain uncertain about its quality.

"The data was ratty, make no mistake about that. But it's there, and it may all be there, all 20 minutes of it, ground up in some scrambled data that was fouled in the communication link between Madrid (one of the three receiving stations for Pioneer transmissions) and California," mission chief scientist Dr John Wolfe said last Thursday.

Late last week, the precise cause of the radio interference was still uncertain. Initially it was stated that the interference had been caused by a Soviet satellite in Earth orbit, Cosmos 1124, which NASA officials had forgotton to ask Soviet scientists to switch off during the period in which the Pioneer data was being transmitted.



"It's the Titans! They say if we want to take pictures we can pay for them!"

On Wednesday, the space agency issued a statement identifying the satellite as the cause of the problem. The statement continued: "NASA officials indicate that they have no doubt that the Soviets would have avoided the conflict on Monday, if they had been asked. However the impact of the potential interference was not recognised in time to make an additional request to the Soviets."

By the next day, however, NASA had changed its mind about the cause of the interference. Rather than the Soviet satellite, they now blamed the combined effect of a solar storm and low quality land transmissions between Spain and California — an explanation which had been put forward at the very beginning, but rejected when the Cosmos satellite's involvement had become known. □

Bulgaria plans to save energy

During the next Five Year Plan (1981-1985), all Bulgarian "energy consuming enterprises" will become subscribers to *Promishlena Energetika* (Industrial Energy), an organisation established this summer and described by Energy Minister Nikola Todoriev as "a scientific production combine". The main purpose of *Promishlena Energetika* is the saving of all forms of energy across the economy; its activities range from the reconstruction and modernisation of existing inatallations to an extensive research and development programme.

What precise form this research would take, the Minister did not specify. He was addressing a symposium on "Fuel and Energy saving and new energy resources" held in conjunction with the annual Plovdiv trade fair. Scientific seminars at the Fair were introduced some ten years ago, and, according to Penko Penkov, Chairman of the Bulgarian Chamber of Commerce, are the response of the organisers to the information explosion.

"Rapid technical progress makes us organise sceintific and technical symposia", said Penkov, "in order to give experts from many countries a chance to keep up with the latest developments". This year, there were no less than twelve day or half-day seminars on subjects ranging from ergonomics, geodesy, and the stimulation of scientific creativity to textile and leather technology and the bacteriology of yoghourt. The three day energy symposium, however, with its massive foreign participation and the full

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panoply of simultaneous translation, undoubtedly dominated the scientific events.

Foreign participation, indeed, was the major feature of the symposium. Apart from the Minister's speech, there were four Bulgarian contributions (on geothermal and solar power, hydrogen fuel and secondary power sources). Otherwise, with the exception of one Polish contribution, all papers were contributed by Western experts, ranging from nuclear to hydraulic power sources, and from general policy to specific devices.

This willingness to borrow Western achievements is nothing new to Bulgaria. One exhibit at Plovdiv — that of the *Progress* organisation, established last year to help bridge the gap from research and development to large scale implementation- carried as its motto a quotation from Mstyslav Keldysh, late President of the Soviet Academy of Sciences, that it is not the nation which makes the first breakthrough who enjoys the benefits of a discovery but the one which implements it properly.

In 1976, said the Minister, when Bulgarian energy policy was revamped with the establishment of the National Energy Complex, Bulgaria had reached the position of one of the most developed nations in the world "in the energy sector at least" having increased specific consumption per head from 9% below world average in 1953 to twice the world average.

The new policy took a three-fold approach, stressing increased exploitation of local resources (mainly lignite), an expansion of nuclear generating capacity (which will reach 35% of the total generating capacity by 1990, with subsequent introduction of nuclear district heating for Greater Sofia) and a major programme of energy-saving.

In spite of massive efforts at economy by industry and domestic consumers alike, these "passive measures" are not considered to be sufficient. Modernisation of existing plants, training of personnel to get the best use from their apparatus, and even the introduction of automated control systems for energy-intensive processes will all peak by the mid 1980s — leaving further gaps to be filled. These, stressed the Minister, can only be satisfied by new technologies.

Some of these will, of course, be of Bulgarian origin. Todoriev mentioned a new Bulgarian scheme, already in operation at the Maritsa-Istok-3 power station, which allows lignite to be burned without predrying — giving an overall fuel saving of some 5%. Others, including solar energy, the use of the geothermal waters of the Rhodope mountains, and the pressing problem of secondary energy resources, may well, if one judges from the Plovdiv seminar, rely considerably — at least in the early stages, — on foreign know-how.

Vera Rich

Pugwash calls for new efforts to control chemical weapons

The Pugwash Workshop on Chemical Warfare has joined impatient voices calling for progress in negotiations on a chemical weapons (CW) disarmament treaty. At a meeting in Stockholm recently, the Workshop urged that the current situation in the negotiations be re-appraised for the Biological Weapons Convention Review Conference, scheduled for 1980. The Review Conference is charged with assessing progress in CW disarmament as well. Most members of the Pugwash group also advocated the reopening of multilateral negotiations on a CW treaty, for there is a major weakness in the Biological Weapons Convention is that it allows research into new biological agents for 'defence'.

Since 1976, the US and the USSR have been having secret talks about chemical weapons disarmament — a subject earlier discussed at the multilateral Geneva Disarmament Conference. But the superpowers' bilateral talks have made no progress. They have instead taken the wind out of the Geneva negotiations, and many of the delegates to the Disarmament Conference have expressed themselves impatient with the stagnation. For example, the leader of the Swedish delegation, Mrs Inga Thorsson, tried recently to move things along when she suggested that the conference should begin its own CW negotiations in parallel with the superpowers. The Pugwash group urged the reopening of multilateral negotiations irrespective of the state of the bilateral talks.

A CW capability consists of different elements: laboratories for research and development, facilities where the weapons are made, and facilities where they are stockpiled. Any treaty aiming at CW disarmament must deal with all these different elements. The Workshop considered them in turn, asking for each whether it would be better to destroy it or convert it to civilian use, and how such measures could be verified.

Pugwash, a private affiliation of scientists interested in disarmament questions, is not an official government body, but it provides a useful arena for its members from east and west to test ideas and atmospheres. The temperature of the official negotiations can be said to be above freezing simply because the Pugwash group is still functioning. At this meeting, the 30 participants produced nothing dramatic; but some of them (who preferred not to be named) were prepared to point to parts of its report with cautious optimism.

It was, for example, said to be a hopeful sign that the report contains a list — agreed by both sides — of six infrastructural recommendations for the verification of the destruction of declared stockpiles. Perhaps the most interesting of these points are that whatever international body is established to verify the destruction, it should have an independent technical capability, and that continuous presence of monitoring personnel at the destruction site would be necessary. The latter does not mean that the USSR, after years of refusing on-site inspection, has decided to allow them after all; but the report reflects agreement on more details of on-site inspection, and in this sense it may indicate a slight softening of the Soviet attitude.

What did emerge, however, from a visit the group made to the Swedish pharmaceuticals factory KabiVitrum AB, is that that on-site inspection need not jeopardise commercial secrets. In recent years, the Workshop has visited commercial chemical factories in West Germany and the United States, and a US Army chemical/munitions disposal facility.

Next year, the group plans to visit a site in East Germany. The visits have enabled the group to explore verification questions which could arise under a CW convention. Invasion of chemical factories by inspectors has long been opposed on grounds of commercial privacy; but as the Pugwash group visits more facilities in more countries, its experience suggests that opposition to some forms of international control of the chemical industry rests on shaky ground.

It was also reported that the discussion on confidence-building measures (CBM's) provoked more reaction than usual. CBM's are steps the sides can take to increase mutual trust: voluntary exchanges of technical and methodological information on chemical research and development related to CW problems, for example. The more trust there is between the sides, the fewer formal verification procedures are needed. Various CBM's have been discussed by previous meetings of the Workshop, but this one produced an additional concept for increasing mutual trust: 'obligatory CBM's under a CW convention'. The group felt that it could be useful to treat certain sorts of treaty provisions as CBM's: provisions, for example, which might be valuable but difficult to verify under a treaty. The discussion on CBM's was laced with controversy about which measures would be best to adopt; and at least one participant was surprised that both sides agreed to mention them favourably in the report.

Wendy Barnaby

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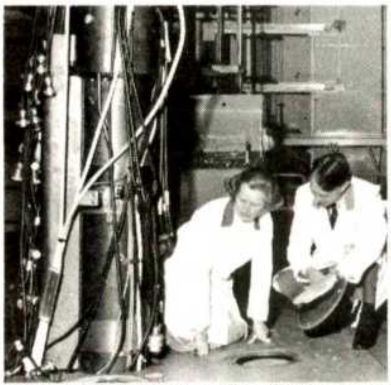
news in brief

Gluons at DESY: Results from the PETRA electron-positron storage rings at DESY, Hamburg, are being taken as confirmation of the existence of the 'gluon'. This massless particle, is according to 'quantum chromodynamics', the mediator of the strong force between quarks: it is the 'glue' which keeps quarks bound into particles such as the proton, neutron, and other hadrons. Quantum chromodynamics (QCD) has been developed in analogy to quantum electrodynamics (QED), the immensely successful relativistic quantum theory of electromagnetism. The gluon in QCD plays the role of the photon in QED. However its discovery may not foretell the 'technological revolution' seen by the *International Herald Tribune* on 30 August: like the quark, it has proved impossible to separate from the hadrons of which it forms a part. The experimental evidence for it, now gathered by several teams at DESY, is a set of events showing the production of particles correlated into three 'jets' or streams — a signature of the decay of a gluon (see *News and Views*, page 104). Early evidence for the gluon was presented by one of the teams at CERN, Geneva, in June; this has been corroborated by data presented at Fermilab, Chicago, by a collaboration headed by Samuel C.C. Ting.



Britain's nuclear future: Britain's Prime Minister, Margaret Thatcher (right), last week reaffirmed her intention to press forward with a nuclear programme for Britain. After starting-up the new fuel reprocessing plant in Dounreay, Northern Scotland, she said that personally she would like to see the go-ahead for a full sized commercial demonstration fast reactor possibly at Dounreay, but there would be a public inquiry first. She expected this decision to be made within two years, and then another eight to ten years to pass before the first commercial station was built.

Britain will also soon have its first pressurised water reactor. The Central Electricity Generating Board has been looking at four PWR designs and is expected to recommend the US Westinghouse design. Sizewell in Suffolk, Dungeness in Kent and Hinkley Point in Somerset are all possible sites currently being investigated by the CEGB.

Meanwhile Friends of the Earth with other anti-nuclear bodies have been discussing ways of improving their effectiveness in the anti-nuclear campaign. According to Czech Conroy from Friends of the Earth, they want a "better coordination of our opposition to nuclear power".

ICRP lung models out: UK National Radiological Protection Board studies published in the Board's latest annual research and development report have shown that industrially produced radioactive dusts do not necessarily behave as predicted by the International Commission on Radiological Protection models. Very small particles of plutonium-239 dioxide, about 0.001 microns in diameter, have been found to be soluble in blood and move rapidly through the body, meaning the radiation dosage to the liver and bone could be up to ten times greater than estimated by the ICRP models. Normally only 0.1% of industrially produced plutonim dust is soluble, but if the plutonium aerosol is produced in conjunction with sodium, for example in fast breeders where sodium is used as a coolant, up to 50% of the aerosol is composed of these small, soluble particles. However, the NRPB studies have also shown that the amount of plutonium entering the blood and deposited in extrapulmonary tissues following inhalation of actinide dusts over 1 micron in diameter, have been overestimated by a factor of ten by the ICRP.

Scientists attack Carter's approval of new missile sytem: The Federation of American Scientists has strongly criticised President Carter's decision, announced at the end of last week, to proceed with the deployment of a new generation of intercontinental ballistic missiles, the MX. After several years of studying alternative proposals for the design of the new system, the President has selected one in which 200 missiles will be deployed in underground shelters, mounted on lorries which can move them between alternative launch sites sufficiently fast to avoid a Soviet missile attack.

The whole deployment programme is expected to cost 32 billion dollars. In its statement, the FAS claimed that the MX "is not just a mistake but a disaster". The federation also voiced fears that the deployment of MX missiles would not only lead to escalation of the arms race, but would also render the Strategic Arms Limitations Treaty untenable, and might result in the abrogation of the anti-ballistic missile treaty. The statement questions the strategic value of the MX — and predicts that it will never be built.

Hungary and UK revise science and technology agreement: Hungary and the United Kingdom have drawn up a new programme for long-term cooperation in applied science and technology. In practical terms, this simply continues existing cooperation projects with one or two additions such as metallurgy and communications engineering. Formally, however, it represents a compromise between the Hungarian and British approaches to such exchanges.

The original UK/Hungarian agreement for cooperation in science and technology was signed in 1967 under the umbrella of the Intergovernmental Joint Commission, which covers all aspects of economic relations between the two countries. In 1977, however, the Hungarians wanted to take scientific cooperation out of the sphere of the Joint Commission, and draw up a special agreement. Exchange agreements between Western and Comecon countries have tended to link trade and scientific exchange probably because scientific cooperation is handled on the British side by the Department of Trade, and on the Hungarian side by a mixed panel representing the relevant ministries. A special agreement could have rationalised this structure, but this very difference made it impracticable to give science and technology an agreement of its own. It does, however, now have its programme formulated in a separate document.

Argentina accused of responsibility for deaths and torture of scientists: The government of Argentina bears "ultimate responsibility" for permitting the disappearances, tortures and deaths of many Argentine scientists, according to a statement issued in Washington last week by the National Academy of Sciences and the American Association for the Advancement of Science. It says that the Argentine government has been "totally unresponsive" to repeated inquiries made into the fates of many scientists, engineers and students who have disappeared since the military government seized power in March 1976.

Such inquiries had been made by both individuals and groups within the scientific community, including the American Physical Society, the American Psychological Association and the Massachusetts Institute of Technology. "The problem is not solved by the government's assertion that it knows nothing of the fate of these 'disappeared' citizens or that they are victims of terrorists," the statement says.

It adds that Argentina cannot have a respected place in the world of international science until the respect for human rights of all citizens permits honest inquiry and freedom of discussion without fear for personal safety.

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Why France has had no public nuclear debate

France has not had the public debates on science and technology of other western nations. **Pierre Papon**, of the Ecole Superieure de Physique et Chimie Industrielles, Paris, outlines why.

ALTHOUGH the past five years have seen considerable — and sometimes violent — political debate in many western countries over the objectives and implications of technological and scientific advances, notably nuclear power and genetic manipulation, France has been remarkably free from public discussion or dissent. The French have traditionally left decisions on science policy to the government in power, and nowhere is this more evident than in the nuclear power programme. Despite its wide socio-political implications, the main decisions on nuclear power have been taken solely by the government, along with a governmental agency — the Commisariat à l'Energie Atomique (CEA) — and the public utility, Electricité de France.

Political decisions have been taken on at least three important occasions in the history of the French nuclear programme. First there was General de Gaulle's decision, based on advice from R. Dauty and the physicist F. Joliot, to found the CEA in October 1945. After the scientific and technological infrastructures had been built up, the parliamentary vote in July 1952 for the first five-year plan for the CEA was a new decisive step. This plan included the building of thermal reactors producing plutonium, which were to form the basis of the future military nuclear programme, launched under the initiative of the then Chief Administrator of the CEA, P. Guillaumat. Although it began slowly, the military nuclear programme accelerated after 1958, and the government eventually decided to shift from gas-cooled reactors to pressurised water reactors. This major decision in 1969 was endorsed five years later when the Messmer government embarked on a new nuclear programme.

Under this plan, the EDF was authorised to build and operate a nuclear capacity representing 38GW of installed power, supplying 50% of elelctricity demand in 1985. This will include 900MW PWR nuclear reactors, and more powerful 1300MW reactors built by Framatome under licence from Westinghourse. The programme will be completed by breeders, the first commercial prototype being the 1200MW Superphenix reactor which is being built at Creys-Malville, Isere, by an industrial society in cooperation with Italy and West Germany. The French nuclear programme is thus extremely important, despite delays and high costs.

Scientists' reactions

After the government had decided to launch this large nuclear programme in 1975, a group of some 400 scientists reacted against the magnitude of the programme and the lack of public debate on nuclear problems. As the 'Groupement de Scientifiques pour l'Information sur l'Energic Nucleaire' (GSIEN) these scientists, mainly physicists, publish regular newsletters giving the main scientific and technical data relavant to the nuclear debate and expounding the socio-political implications. Since it has political contacts and an honest audience, this group has performed a useful task in disseminating information. The union of workers of the CEA, affiliated with the CFDT, has regularly warned the authorities of the technical and social risks of the nuclear programme; recently for example, they drew attention to safety problems related to working conditions at the fuel reprocessing plant in La Hague. The action of the physicists in promoting a public debate on nuclear problems, however, has no equivalent in other fields: a group of biologists, among them a few connected with Pasteur Institute, met with little success when they tried to initiate a public debate on recombinant DNA research a few years ago.

The Three Mile Island accident has recently put increased emphasis on the question of nuclear reactor safety. The French Physical Society (Societe Francaise de Physique, SFP) the professional society of physicists, seized this opportunity for opening a debate on the problems of reactor safety at its biennial general meeting in Toulouse in the last week of June. The discussion now conducted by a panel of physicists, which included two members of the CEA (M. Tanguy, Head of the Protection and Nuclear Safety Institute and M. Droulers, Director of the Department of Evaluation of Safety of Reactors) and was chaired by B. Dreyfus, vice-president of the society. The members of the CEA were questioned on the Harrisburg accident and on its implications for the French nuclear programme, especially for the safety of the PWR reactors. The debate focused mainly on technical and scientific problems, such as the behaviour of the primary coolant in biphasic conditions, the crucial problem of instrumentation inside the containment vessel, the reliability of data available to the operators, and the probability of major accidents like those occurring after a thermal shock or a core melting. Questions were also asked about the technical competence of reactor operators and their ability to cope with a major accident.

The participants also tackled the question of whether outside scientists should be involved in planning and executing nuclear safety research programmes. The concensus was in favour, but no precise agreement was reached on how such participation could be achieved. The CEA physicists did display confidence in the PWR reactors, although they admitted that some events which occurred at Three Mile Island had not been taken into account in considering accident risks, including the formation of a large hydrogen bubble, and they also conceded that more attention should be paid to the behaviour of the secondary system and to the instrumentation of the primary coolant.

Although this meeting is not a landmark in the nuclear debate, it is important in that it constitutes the first attempt by the Societé Francaise de Physique to open a discussion among its members on an important element of the nuclear debate. Other discussions, on the fuel cycle and on various reactor systems (including breeders), are planned.

Government traditions

The reasons for the lack of previous public debates on major technological issues like nuclear power lie largely in the traditions of French government. Until the second world war, successive governments held science and technology to be low priorities in national policy, although they kept a tight hold on some industrial areas like energy supply. With the post-war move into high technology, state intervention took place in research areas important to France's technological independence, beginning with nuclear power in 1945, and extending to aerospace and computers in the 1960s.

Because of their national strategic importance, decisions in these fields have been felt to be the responsibility of governmental decree rather than laws passed by parliament.

In the nuclear power field, there have been a few debates in parliament, and no general legislation has been passed. For example, although the breeder Superphenix is partly financed through the budget, the decision to build such a reactor was taken by a decree in May 1977 without a vote in parliament. France does not have any procedure of public inquiries to allow the assessment of major technololgical programmes with the participation of independent experts, as in the Windscale inquiry in the UK. These two facts have combined to weaken the possibility of a serious political debate.

Parliament's role is a rather weak one, since it has no standing committee on science and technology as such. The 'Commission des Finances' deals with major problems of science and technology in the National Assembly, but these two committees do not have sufficient technical means to investigate complex technical problems. In April 1979 a law creating a

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special permanent comittee for the assessment of major technical proposals was passed by the National Assembly — against the opposition of the Minister for Industry A. Giraud (a former head of the CEA), who declared it unconstitutional; it is now before the Senate.

After the Harrisburg accident a special investigation committee was set up by the National Assembly and its four members went to the US. In their preliminary report, published on 27 June, the deputies insisted on the necessity of informing the public about nuclear safety, and they insisted that the government should publish the intervention plans prepared in the case of an accident. This report, although useful, falls short of the full-scale assessment needed of the safety problems involved in the nuclear programme.

In France it is also traditional that the government resists efforts to make public all the factors on which its decisions are based. In the energy domain the weight of the powerful public organisations EDF and CEA is not balanced by other institutions challenging their conclusions.

The high-ranking Civil Service considers that it has, as the ultimate political power in the country, the right to intervene in high technology sectors and to control the decision-making process. Information also comes under its power, and it is reluctant to abandon even some of this to parliament, which has indeed rarely shown a real interest in the problems of science and technology. The creation of a special inter-ministerial committee, chaired by Mrs Simone Veil, former Minister of Health, which will disseminate information on nuclear energy is a step in the right direction. Unfortunately this committee has devoted most of its time, since its creation more than a year ago, to simply informing itself.

Another element of the problem is that the French government usually trusts what L. Kowarski called 'les competences instituées' (representatives of public institutions, business, large organisations, and so on). It is typical that the government asked a panel of members of the Academie des Sciences to comment on the official report of the Harrisburg accident; and, given the reluctance of the Assembly to engage in controversial matters, one can estimate how useful their opinion will be.

Competent experts who may have views and opinions which conflict with those of the government are not involved in the decision-making process. To be fair one must add that the scientific community, with few exceptions, has rather fought shy of all problems related to the implementation of science and technology. It has tended to expound its views on the general situation of research, and retreated from questions with social or political overtones. One may hope that the severity of the social and economic crisis will now oblige the government and the scientific community to enter a dialogue with public opinion. ☐

French scientists oppose CNRS reforms

A STORM is building up within the French scientific community around the government's proposed reform of the country's highly centralised research organisation, the Centre National de la Recherche Scientifique (CNRS). In August nearly 200 scientists, engineers and technicians demonstrated against the plan — and this in a month when nearly all the French go on holiday. In addition, a lengthy polemic has been opened in the national press on the subject while the main trade unions involved in the CNRS are promising further activity this month.

The reforms, proposed by Giscard d'Estaing's government, have two basic themes: a structural reorganisation of the CNRS itself and a change in the direction and control of research goals. The remit of the CNRS runs from pure mathematics through physics, chemistry, biology to linguistics, musicology, history and philosophy. Thus any change is bound to have a major impact. It was originally set up to develop the career of "research scientist" independent of university teaching and as a profession in its own right. Currently its budget is FF3,500 million (more than £35 million); it employs 8,300 research scientists (more than one half the national total) and 13,000 engineers, technicians and clerical staff, all its employees enjoying the rank of civil servants.

The CNRS is presently governed by a rather Byzantine array of interlocking committees and directorships. The original plan envisaged a system with most committees at the top having both elected and appointed members. Thus, for example, the National Committee for Scientific Research, which is supposed to oversee part of the budget and the performance of the various laboratories, is composed of 41 subcommittees, each covering a seperate discipline, and each with 26 members — 15 elected by the staff and 11 named by the government.

In fact another more hiearchial structure has grown up within the CNRS. It consists of seven directors, each in charge of a commission which oversees the work of all labs in its area. The directors report to the director-general of the CNRS, currently M Robert Chabbal. There are no elected members in this chain which has now established *de facto* control of finances and the review of the work of the centre. In the words of M Chabbal, "the French like pyramidal structures."

The first part of the proposed reforms will "regularise" this unofficial structure, giving it permanent form and official control. Although the National Committee would continue to exist it would have only advisory powers, while real power will lie with the appointed directors and their commissions. The main thrust of this part of the reform then is to introduce a more "streamlined" though less "democratic" management with more autonomy for the different fields of research.

The three unions, who represent a substantial number of the scientists, engineers and technicians at the CNRS, and whose members took part in the August demonstration, are calling for militant action against the reform. Meanwhile a petition circulating among researchers at the CNRS had gathered 230 signatures within the first fortnight of its launch.

The second aspect concerns the structure of research itself. The traditional "vertical" division of the CNRS by discipline has for some time been supplemented by "horizontal" intra- or inter-disciplinary projects. Examples of these are the 30 or so GRECO (Groupements de Recherches Coordinees) agreements which reflect national level single discipline studies and the more than 40 GIS (Groupements d'Interets Scientifiques) or cross-disciplinary studies, for example, the CNRS/University of Montpelier/local authorities solar energy project. The reform calls for an increased emphasis on these "horizontal" structures with more input and responsibility being given to the CNRS's partners, particularly industry.

What is feared is that the real aim of this reform is to dismantle the centralised structure of the CNRS with forcible merging of laboratories, "nationalisation" of the work force and the placing of the resulting smaller and more vulnerable pieces under private industry. In particular, the social and human sciences are scarcely visible in the new scheme while the more "physical" disciplines will be heavily weighted towards rapid and easy profitability.

On the one hand, the director-general M Chabal, stresses the advantages of more fluid discipline boundaries and increased ease of formation of new research areas. On the other, opposition scientists and unions warn of private industry's control of research goals and the loss of autonomy of the scientific community. Whether a major confrontation will actually develop depends largely on the government's tactics. In France, it is not uncommon for proposed legislation to be postponed even after its announcement or for bills, once passed, to be left unenforced.

If the reforms go ahead one result may be the opening up of debate on the nature of research itself, which is beginning to be discussed in the socialist trade union CFDT. Are scientists, private industry and the State the only possible judges of the value and direction of research? What about the people themselves and their participation in research decision?

Jim Ritter

Jim Ritter is in the Department of Mathematics, King's College, London.

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Nuclear India

This week a law comes into operation in the US which will have the effect of foreclosing nuclear fuel supplies to India. **Anil Agarwal** here considers India's nuclear future

India's underground nuclear test in Rajasthan: Desai condemned it

THERE is no more emotive tissue in the field of science and technology in India today than atomic energy. Since the 1974 nuclear test at Pokhran, the Indian Government has been under enormous pressure from western nuclear powers — in particular US and UK — to sign the Non-Proliferation Treaty (NPT). India has always insisted that the Pokhran test was entirely intended for peaceful purposes and that the NPT is an extremely discriminatory document, which does nothing to curtail the proliferation of nuclear weapons in the existing nuclear weapon states.

While all Indian governments had regularly denied any interest in nuclear weapons, Prime Minister Morarji Desai turned the making of any nuclear bomb by India into an even stronger moral issue than had his predecessors. In characteristic style, Mr Desai told western reporters after the Commonwealth meeting in London in 1977: "I'll give it to you in writing that we will not manufacture nuclear weapons. Even if the whole world arms itself with the atom we will not do so". Recently, when the news broke that Pakistan was involved in building a uranium enrichment plant, there were demands from several members of parliament that India should make its own nuclear weapons. But Mr Desai again stated: "I am neither prepared for the manufacture of the atom bomb nor will I sanction funds for it".

When Mr Desai was Prime Minister, he closed Indian options for peaceful nuclear explosions, which all earlier Prime Ministers had studiously kept open. He even criticised the Pokhran explosion, which had been proclaimed a major national achievement during Mrs Gandhi's term in office — a view shared by most of the country's intelligentsia.

Mr Desai told parliament that Mrs Gandhi had conducted the Pokhran explosion for political rather than scientific gains. Mr Desai immediately faced a barrage of criticism. "Your unilateral declaration abjuring nuclear explosions even for peaceful purposes and ambiguous and contradictory statements on nuclear policy have raised profound misgivings about the future scope and objective of our nuclear programme," wrote an angry Mr Y.B. Chavan, in a letter to Desai in late 1978. Mr Chavan, a veteran Indian politician, who was then leader of the Opposition and is now Deputy Prime Minister in Mr Charan Singh's government, added: "we were very surprised and disturbed by your extraordinary statement on the Pokhran explosion. You may have had your own reasons for making it, but in the process you chose to pronounce on the technical aspects of the explosion, denigrating our scientists and engineers who were involved in the project. It has been recognised by experts all over the world that technically the Pokhran explosion was a fine job.

Mr Desai replied reiterating his stand. "It is quite clear to me" he wrote, "that you do not know the details and the lessons that were learnt. Technically there is no doubt that it was a good effort but compared to achievements elsewhere it was a very small effort." Moreover, the explosion had created international problems for India. He explained that the "explosion is always held against us as a preparation for warlike use of atomic energy and you know very well how it is being exploited by our neighbours so that they can blackmail foreign governments into assisting them in acquiring that capability themselves."

With foreign nuclear powers, Mr Desai took a straightforward moral position. He agreed to throw open all the nuclear reactors in India to international inspection and accept fullscale safeguards, but only on the condition that the nuclear powers themselves sign a comprehensive nuclear test ban treaty, agree to reduce their own stockpile and open their own reactors to international inspection. Mr Desai informed Mr Chavan in his letter: "In my private discussions both with President Brezhnev and President Carter I have even made it clear that unless the safeguards are extended to military reactors they cannot insist on our having them."

When Mr James Callaghan, the then UK Prime Minister, followed President Carter to negotiate with Mr Desai, just a week after the latter's visit to New Delhi in early 1978, Mr Desai once again refused to sign the NPT. But he agreed to open India's reactors to international inspection once there was a basic international agreement amongst UK, USSR and USA for a comprehensive test ban treaty. By leaving out France and, more important, China, which is the immediate nuclear power on India's borders, even several western observers accepted that Mr Desai had made a significant concession. Earlier this year, President Carter publicly admitted that he found it difficult to argue with Mr Desai's moral position.

However, within India itself Mr Desai was strongly criticised by almost all leading newspapers and politicians for his soft and yielding stand over NPT. Many commentators said that he had set India on a slow march towards acceptance of international safeguards. A leading columnist of the Times of India, wrote: "Mr Desai is a man of high principles. He is a conscientious objector to nuclear weapons under any circumstance. It is to his credit that he would rather resign than do anything against his conscience. Even so, must he forswear, on behalf of India, nuclear weapons for all time to come, regardless of the circumstances? As a senior member of (former Prime Minister) Mr Nehru's cabinet, he must know that it was with the full concurrence of the then Parliament that Panditji had declared himself to be opposed to going in for nuclear weapons. But he had taken care to add that though he hoped that future parliaments would agree with his view, he would not bind them down. This is a more rational approach than a blanket rejection of nuclear weapons. And the least that the Prime Minister ought to do is to seek the present parliament's endorsement for his stand."

When the Indian government agreed to a US proposal to set up an independent committee of nuclear experts to study, as Dr Jospeh Nye of the US put it, "the question of what safeguards options were available for states which were not members of the NPT", there was immediate suspicion amongst politicians and press commentators that the country may have walked into a trap. The reasons for setting up the committee have never been sufficiently explained but it seems US diplomats had claimed that the committee's deliberations would help to soften public opinion in the US against the supply of nuclear fuel to India.

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However, until the western powers made up their minds about a comprehensive test ban treaty, Mr Desai's government took careful steps to ensure that India does not have to accept fullscale safeguards on its reactors. The constant irritant in Indo-US nuclear relations is the Tarapur Atomic Power Station. The US government is obliged under a 1963 agreement to supply enriched uranium fuel for the entire lifetime of the station. But under a new law, it now has to stop supplying nuclear fuel to any country which has not signed the NPT by March 1980. The law does not even exempt sales to those countries with whom the US had previous agreements. Naturally, the Indian government has protested against it.

Over the past few years there have been extraordinarily long delays in supplying nuclear fuel to India. The last consignment of fuel arrived in March, 1979, about 13 months after the Indian government had applied for it. At that time, the US government had not even presented to the Nuclear Regulatory Commission, which permits sales of nuclear fuel, another Indian application sent in September 1978 for yet another outstanding consignment. Many Indians felt that the March consignment too had been sanctioned only because Indian leaders had started discussions with the USSR over the supply of enriched uranium. But because of these delays, the Nuclear Fuel Complex at Hyderabad, which manufacturers fuel elements, had to close down its workshops. The new US law forbids the government from considering further applications for nuclear fuel sales after 10 September.

The US government had believed that the new law would force the Indian government to see its viewpoint. But Mr Desai, strongly supported by Indian public opinion, informed the US government that refusal to supply enriched uranium for the Tarapur reactors would be treated as a unilateral abrogation of the previous agreement. India would have no further obligations to observe the bilateral safeguards which are imposed on the reactors at the moment and would be free to reprocess the spent fuel from them. Under the existing agreement, the US has the right to buy back the fuel.

Plans to use plutonium

No decision about the spent fuel has yet been taken and it is being stored in reservoirs which are now full. The storage capacity is to be expanded, but as soon as the legal position about the fuel is clear, India would like to extract plutonium from it at its waste processing plant at Tarapur. The Indian Atomic Energy Commission claims to have developed a new fuel consisting of natural uranium enriched with plutonium to replace the enriched uranium fuel supplied to the US.

Scientists at the Bhabha Atomic Research Centre have indigenously designed and built a plutonium-fuelled small experimental reactor so India has experience of working with plutonium fuels. With fuel supplies that have already been agreed upon, the Tarapur station should be able to generate power until 1982. That should give the Atomic Energy Commission time to fabricate the new fuel.

The option of using mixed plutonium and uranium oxide fuels could embarrass the US government which has always been against the use of plutonium in thermal reactors, and has even tried to prevent European countries from using it. Its diplomatic offensive against India might well produce a situation it has otherwise tried to prevent. Once plutonium starts being used in thermal reactors, the total amount in the world could jump from 25 tonnes to well over a thousand tonnes, making it almost impossible to police its use. The fuel supply for Tarapur is, therefore, supposed to be an open issue. The US President may well decide to continue to supply nuclear fuel to India to stop this precedent from taking place. There is, of course, the even more important worry that Indo-US relations would take a nose-dive.

The Desai government had also taken steps to prevent fresh safeguards being applied to the Rajasthan Atomic Power Station (RAPS). After the Canadian government had unilaterally broken its agreement with India in 1976 to supply heavy water for the second unit of RAPS, Mrs Gandhi's government had started negotiations with the USSR. The Russians, however, insisted on even stronger multiple-point safeguards than the Canadians. An agreement with the USSR was concluded in 1977, soon after Mr Desai's government took office. Under it the IAEA has the right to inspect any other nuclear system in which any material generated in the Rajasthan station is used. India has an experimental fast breeder reactor under construction at Kalpakkam, where its second major nuclear research centre is being developed. RAPS-II is now reported to be ready but the Russian heavy water has not yet been used. Until it is, the safeguards agreement does not come into operation. It is possible that India will keep the RAPS-II idle until it can manufacture its own heavy water.

Apart from the Tarapur station, all the Indian stations are heavy water-moderated, natural uranium-fuelled, CANDU-type reactors. India manufactures its own natural uranium fuel, but remains critically short in its heavy water requirements, and is thus dependent on foreign sources. Apart from a small heavy-water production unit in operation at Nangal, there are four large plants under construction. The first is being built by Indian engineers at Kota. Designs for this plant were obtained from Canada but even the Canadians have found difficulties with similar plants. The Kota plant too has been plagued with difficulties and delays, and has thus seriously undermined India's plans to gain expertise in heavy water production. It is expected to begin operation in 1980.

Three other plants are being built at Baroda, Talcher and Tuticorin by French and West German companies. All are suffering delay. The Baroda plant started trial runs several years ago but a chemical explosion seriously damaged it and it is now being repaired. The Talcher plant was delayed when two massive steel towers which had been shipped from Europe got mysteriously swept away at sea. Both the Baroda and Talcher plants are now expected to start operating by the end of this year. The Tuticorin plant has been undergoing trial runs but it has been plagued with problems in the ammonia plant of the fertiliser factory with which it is linked. Once these plants begin operating, they should be able to supply the heavy water needed for the nuclear reactors under construction.

The Indian nuclear power programme itself has been lagging behind the schedules projected in the early 1970s because of serious management and construction problems. Nuclear stations at Madras and Narora, which are under construction, are expected to take more than ten years to build, and their costs have escalated considerably. This has led to resistance from both the Ministry of Energy and the Planning Commission to sanction funds for more nuclear reactors. This situation existed under Mrs Gandhi's government but Mr Desai told parliament that the government wanted first to cope with the atomic power plants already taken up before thinking of setting up new ones. A fifth plant has been under consideration for several years — either in the State of Gujarat or as an extension to the existing Tarapur Station. Mr H.N.Sethna, Chairman of the Atomic Energy Commission, claimed a few weeks ago that its location may be decided by December.

The slow progress of the nuclear programme has certainly left young engineers working with the Atomic Energy Commission in a very uncomfortable situation as far as their careers are concerned. The plans formulated in the early 1970s had envisaged that one nuclear power station would be sanctioned every year. But now many senior engineers have remained stuck in their old positions for over a decade as they have no new sites to move to, and their junior colleagues have remained in their junior positions. It is the same story, for different reasons as in many developed nations. But the under-employment of engineering talent — some 30,000 working in India atomic energy establishments — is a much more severe problem in a developing country. The unfortunate thing is that as long as external foreign pressures exist, no government in India will find it easy to evaluate seriously the role of nuclear energy in its future energy development programmes. □

correspondence

Biased reporting of East European science

Sir, — There should be little doubt nowadays that science needs extensive, systematic and friendly international ties for its own progress, for positive service to humanity and for contributions that such ties make toward safeguarding peace and avoiding the world-wide devastation which advanced science and technology have guaranteed to be inherent in a war between major powers. Even arms limitation and, hopefully, arms reduction are not only general human concerns but also professional issues affecting the direction of science and the question as to whether its major efforts will be oriented toward destruction or toward human welfare.

The near catastrophe this year at the nuclear plant at Three Mile Island, Pennsylvania, has underscored dangers which could arise even in the absence of war, and leads one to be grateful for the existence, since 1973, of the US-USSR Joint Committee on Co-operation in the Peaceful Uses of Atomic Energy. Its fifth meeting (Washington, April 1978) gave prominent attention to work on 'Light-Water Reactor Safety' — the type of reactor used in the US — and to plans for intensifying work on this topic, whose importance the whole world now appreciates.

A sense of responsibility to science, to peace, to mankind would require the encouragement of such co-operation and its substantial expansion.

It is, therefore, very sad to observe *Nature*'s failure to exercise this responsibility, and its degeneration into a propaganda sheet for those anxious to whip up an hysterical ill-will against East European science. In what has assumed the dimensions of a campaign, it seems to be doing what it can to encourage those seeking to destroy co-operation, or even contact, between the scientific communities of Eastern Europe and those of the US and its allies. News articles do not conceal their bias and editorialising. For example, a news report (7 September 1978, page 3) under the headline "Boycott of Soviet contacts is for individuals, says NAS [National Academy of Sciences]" stated early on "This reaction would appear to substantiate the case for boycott, a case most ably put by Valentin Turchin". The rest of this 'report' is similarly tendentious and argumentative. Incidentally, the Turchin letter and similar ones were published, not in the space normally reserved for letters, but more prominently, in larger type and with conspicuous headlines.

The author of that article is a regular contributor to *Nature*, Vera Rich. Her numerous articles are chiefly propagandistic, lacking in analysis, highly selective, crowded with sneers and sarcasm, ill-mannered and disruptive, and contemptuous of Soviet science. They do not offer the reader any well-rounded picture of East European scientific life and developments, to say nothing of the fruits of international scientific cooperation.

Underlying all the sarcasm and the jibes is the unwarranted assumption that Soviet science and the science of other socialist countries is very much the junior partner in any cooperation. Occasionally this is stated explicitly, and such views given more or less unchallenged publicity. One anonymous letter (14 December 1978) asserts that in 1965 the US and the USSR had about the same number of scientists, but that the former did one-third of world science, the latter only one-sixth. Leaving aside measurement problems, one might ask why *Nature* didn't point out that through its money the US has fortified its science establishment by visiting upon other countries a 'brain drain' which has caused even its closest allies to complain and which has damaged developing countries, while the USSR has relied almost exclusively on its own population. As a perhaps atypical example, it might be noted that the School of Mathematics of the Institute of Advanced Study at Princeton has normally been staffed at the professorial level by a majority of great imports.

In any case, Soviet and other socialist scientists are no 'junior partners' in international science, no matter how *Nature* portrays them.

But the blame for this destructive campaign cannot be placed on Vera Rich's pen alone. *Nature*, itself, has weapons in its arsenal. Some day a doctoral dissertation will analyse headlines, positioning, type-sizes, linguistic techniques and much else, to the amusement of the author and the embarrassment of the editor.

Without awaiting such refinements, I am concerned about such things as an *anonymous* full-page article (13 October 1977, page 548) by what *Nature* calls "a correspondent with some experience of East European countries, . . . [who] returned [from the GDR] with these impressions". No explanation is given as to why this correspondent was permitted a cloak of anonymity to shelter an ungracious guest's snide barrage. *Nature*'s editorial introduction to this piece declared "Scientific exchanges with the German Democratic Republic (GDR) are rare, and our knowledge of the state of its science is consequently sparse". It neglected to mention that NATO countries refused until recently to recognise the GDR, excluded it from the UN, and were unwilling to negotiate exchanges earlier, or even to permit GDR citizens to visit under their own passports. Every effort was made by NATO to isolate the GDR. For years the International Mathematical Union allowed its FRG-affiliate to act for both German States.

In passing, let me mention that I have made three brief scientific visits to the GDR (in 1965, 1972 and 1978), and that the colleagues I met there were well-trained, skillful, enthusiastic scientific workers who were far from conveying the "frustration and pessimism" which your anonymous correspondent claimed to be the prevailing mood.

I hope that the editors will not await future doctoral candidates to analyse the myriad ways in which *Nature* demeans itself today. *Nature* is an important voice in the international scientific community. Its editors should take cognizance of the grave responsibility this implies, and should bestir themselves to establish some objectivity, balance and reason into its activities impinging on the crucial field of scientific co-operation between the countries of Eastern Europe and other major scientific centres.

Yours faithfully,

Lee Lorch

York University, Ontario, Canada.

Uranium use in fast reactors

Sir — R.D. Smith in his letter on fast reactor safety (23 August, page 630) says, "remember the fast reactor will produce about 60 times more energy than a thermal reactor for the same quantity of uranium".

I thought this particular piece of nulcear nonsense had been scotched for good. It might be true in 200 years time, but certainly is not for any period which is significant for our present situation. When challenged by Leslie Granger and David Merrick (*Energy Policy* December 1976, and *Nature* 16 December 1976, pages 596-8) Bart Cutts, Head of Technical Services at UKAEA eventually (*Energy Policy* September 1977) presented a "notional programme of electrical installation". One is justified in assuming that it was the best case he could produce, especially as he found it necessary to assume a very low growth rate for electrical energy of 2½% p.a. Even so the ratio of utilisation of uranium for a programme based on the maximum development of FBRs and one based only on thermal reactors was 1.1 by 2000 and only 1.7 by 2020, compared with this absurd figure of 60 times which might be achieved by 2200.

For the sort of increases in electricity production that used to be achieved and were projected at least until 2000, i.e. anything over 5½% p.a., Granger and Merrick have shown that the ratio cannot exceed 1½ times as long as this rate of increase continues. The reason is simply that, as Walter Marshall has said, fast breeder reactors are really breeder fast reactors, or perhaps better, slow breeder fast reactors. To achieve even the rate of increase, modest by historical standards, of 5½% p.a. it is necessary to build large number of thermal reactors to provide most of the increase and furnish plutonium to start up the FBR series. These gradually breed more plutonium, and the process goes asymptotically to the use of 1½% of the uranium instead of the 1% for thermal reactors. The higher the annual increase is above 5½%, the more slowly is this figure of 1.5 approached, never to reach any higher unless the annual increase comes below 5½%. As we have seen, even for an annual increase as low as 2½% p.a. it only goes to 1.7 by 2020.

On a related point, in order to reach a stage when further supplies of uranium are no longer required, the rate must be below 3% p.a. Above that the need to import uranium never ceases.

It is to be hoped that R.D. Smith's corrections of Norman Dombey (other than the misprint) are rather more relevant than his reference to the improved utilisation of uranium.

Yours faithfully,

J.W. Jeffery

Birbeck College, University of London, UK.

Defending science and rationality

Sir, — I have just read your editorial (23 August, page 619), on 'Science, nonsense and responsibility'. I do so with that slightly sour amusement always induced by the spectacle of Satan denouncing sin. Having read your editorial and review columns for many years, I find your pose as a defender of science and rationality decidedly unconvincing. However, if you should now switch to opposing the major as well as the trivial superstitious movements of our time, no one would be more pleased than I.

Yours faithfully,

M. Hammerton

University of Newcastle upon Tyne, UK

news and views

Structure and force generation in muscle

from John M. Squire

THE sliding filament model of muscular contraction, proposed 25 years ago, is now generally accepted to account for the shortening in muscle; the thick myosin filaments interdigitate with the thin actin filaments and when these filaments slide past each other the muscle changes length. However, within this basic scheme, relatively little is known about the structural details of the force-producing mechanism which causes the filaments to slide. The thick filaments contain projections (the myosin heads or crossbridges) which can split ATP, which can bind to the neighbouring actin filaments and which have an ATP turnover rate that is increased by actin binding. Tension is related to ATP usage, so the crossbridges are clearly implicated in force generation. It is commonly assumed that the crossbridges in an activated muscle first attach to actin and then change their attachment configuration through a number of mechanical steps during which force is generated. The crossbridges subsequently detach from actin and reset themselves ready for a further attachment cycle. Although the kinetics of the biochemical steps in the actomyosin ATPase cycle are fairly well defined (see Lymn *A. Rev. Biophys. Bioeng.* **8**,145; 1979) the details of the corresponding changes in structure are still relatively uncertain and much effort is being devoted by structuralists to defining the sequence of mechanical steps involved in force generation.

One of the most useful experimental probes available to the structuralist is the X-ray diffraction technique, since, unlike electron microscopy, it can be applied to muscle which is living and, if required, actively producing tension. Of the various regions of the resulting X-ray diffraction patterns which can be studied, one of the most informative is the equator. This is the region which provides information about the density profile of the muscle when viewed down the muscle axis. Figure 1 shows schematically the appearance of such a view in the A-band of vertebrate skeletal muscle. Here the myosin filaments (M) form a hexagonal array with the actin filaments (A) at the trigonal points of the lattice. The principal diffracting planes in this lattice are the $10\bar{1}$ and $11\bar{2}$ planes as indicated in Fig. 1. The actin filaments lie between the myosin filaments when the assembly is viewed along the $10\bar{1}$ planes, but are in line with the myosin filaments in a view along the $11\bar{2}$ planes. This means that if there is a movement of mass from near the myosin filaments to near the actin filaments when a muscle is activated, the effects on the $10\bar{1}$ and $11\bar{2}$ X-ray reflections will be different. More mass at the actin filaments will tend to smooth out the density profile in a view down the $10\bar{1}$ planes (that is, the $10\bar{1}$ reflection will get weaker), whereas it will tend to enhance the density profile in the $11\bar{2}$ view (that is, the $11\bar{2}$ reflection will get stronger). In fact it was shown very clearly by Huxley (*J. molec. Biol.* **37**, 507; 1968) and later by Haselgrove and Huxley (*J. molec. Biol.* **77**, 549; 1973) that the intensity ratio ($I_{10\bar{1}}/I_{11\bar{2}}$) of the $10\bar{1}$ and $11\bar{2}$ reflections changed from about 2.5 for muscles at rest, to slightly less than 1 for muscles generating tension and to about 0.25 for muscles put into rigor (all at rest length). The rigor state is known to be one in which a large number of crossbridges are attached to the actin filaments and these observations were taken to mean that fewer crossbridge attachments occur when a muscle is generating tension than in rigor.

Unfortunately, although it is generally true that the more attached crossbridges there are the lower will be the ratio $I_{10\bar{1}}/I_{11\bar{2}}$, the attachment number is in theory only one of a number of factors which can affect this ratio. Lymn showed (*Biophys. J.* **21**, 93; 1978) that the configuration of the attached bridges also has a marked effect on this ratio. Lymn considered two attached states for the myosin heads, one with the long axis of the heads at about 45° to the thin filament axis (as in the rigor state) and one at about 90° to the thin filament axis. His modelling showed that a ratio $I_{10\bar{1}}/I_{11\bar{2}}$ of about, say, 0.5 could be due either to labelling by crossbridges in the 45° configuration of 40% of the actin monomers in the thin filaments or to 20% labelling by crossbridges in the 90° configuration. Since it is now thought that a number of attachment configurations might be involved in each crossbridge cycle in active muscle, it is clear that the intensity ratio of the equatorial reflections will not in general be a good indicator of the number of attached crossbridges. However, provided the distribution of crossbridges among the attachment states is constant, as may well occur in muscles which generate tension at a fixed length (isometric contractions), it is possible then that the equatorial intensity ratio might vary directly according to the number of attached crossbridges and therefore, on present thinking, to the level of isometric tension generated by the muscle. In a recent

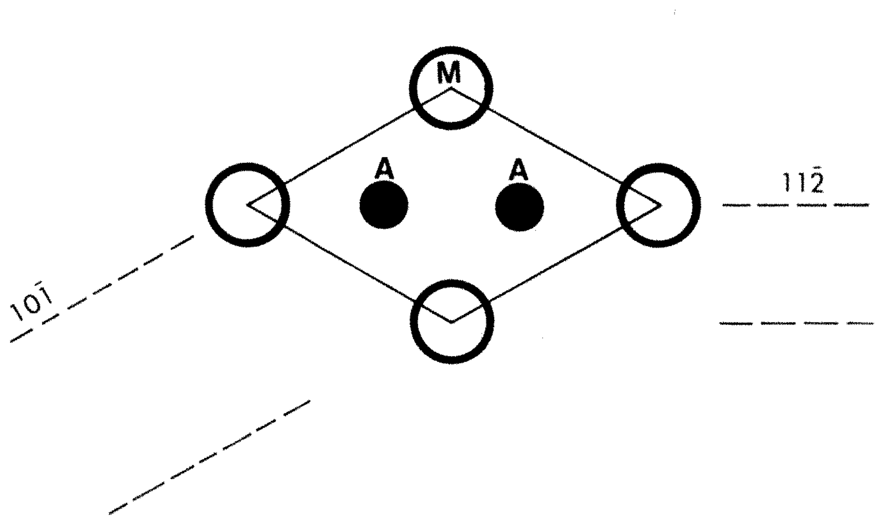

Fig. 1. Unit cell of the hexagonal array of filaments in the overlap region of the sarcomere in vertebrate skeletal muscle. M, myosin filaments; A, actin filaments. The directions of the $10\bar{1}$ and $11\bar{2}$ planes are indicated, in this view of the unit cell down the long axis of the muscle.

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paper, Yu, Hartt and Podolsky (*J. molec. Biol.* **132**, 53; 1979) have presented the results of an elegant series of X-ray diffraction experiments in which they have monitored the individual intensities (and the intensity ratio) of the $10\bar{1}$ and $11\bar{2}$ equatorial reflections from frog sartorius muscles producing different levels of isometric force. They found that the ratio $I_{11\bar{2}}/I_{10\bar{1}}$ (the reciprocal of the ratio discussed above) increased systematically with increasing force (with a slight upward curvature), while the individual intensities either decreased ($10\bar{1}$ reflection) or increased ($11\bar{2}$ reflection). The observed variations were said to agree well with theoretical curves calculated assuming two populations of crossbridges, attached and detached, and the attachment number increasing linearly with tension. Although this is clearly an oversimplification, it supports the general contention that more crossbridge attachment should be associated with greater tension generation. This result differs markedly from the previous observations of Podolsky *et al.*, (*Proc. natn. Acad. Sci. U.S.A.* **73**, 813; 1976) on muscles shortening at different speeds under a constant load. In this case it might be expected that both the attachment number and the distribution of attached states would vary with shortening speed, but Podolsky *et al.* found that the equatorial intensity ratio varied very little. It must be concluded either that the current ideas are wrong or that both the distribution and number of the attached bridges change together in such a way that the effect of one is virtually cancelled by that of the other.

One feature of the new results of Yu *et al.* on isometric contractions, not immediately explained by their modelling, was the observation that the intensity of the 101 reflection dropped rapidly from its resting value even at relatively small tensions and then reduced more slowly as tension increased. Yu *et. al.* attributed this to some disorder in the filament lattice accompanying the low level of crossbridge attachment, but an alternative addition to the simple two state model is the addition of a third activation step before crossbridge attachment to actin can occur (Squire *A. Rev. Biophys. Bioeng.* **4**, 137; 1975). Such an activation step is suggested by X-ray diffraction results from muscles stretched so that no filament overlap occurs (Huxley *Cold Spring Harbor Symp. quant. Biol.* **37**, 361; 1973; Haselgrove *J. molec. Biol.* **92**, 113; 1975). Here evidence was obtained for changes in thick filament structure on activation of the muscle even though no actin attachment was thought to be possible.

But the detailed interpretation of these results depends critically on the symmetry of the myosin filaments in vertebrate muscle. The crossbridges are thought to be arranged on a multi-stranded helix around the cylindrical myosin filament shaft. A 3-stranded model is now generally accepted but recent biochemical evidence (Pepe & Drucker *J. molec. Biol.* **130**, 379; 1979) suggests that there may be four strands. However, convincing structural evidence is required to settle the matter finally. Nor is this problem confined to vertebrate muscle. The thick filaments in insect flight muscle, larger in diameter than those in vertebrates, were initially thought to be 2-stranded and then 6-stranded. Now, Wray (*Nature* **277**, 37; **280**, 325; 1979) has suggested, largely on circumstantial evidence, that the number of strands of crossbridges on the insect thick filaments might really be four. It is crucial to our understanding of the mechanism of muscular contraction that the symmetries of these filaments be determined unambiguously. It would then be possible to interpret sensibly the wealth of X-ray diffraction data already obtained from muscles in a variety of states. The known biochemical kinetics of the actomyosin ATPase could then be related more realistically both to the number of attached crossbridges and to the different structural steps involved in the crossbridge cycle. □

John Squire is a Lecturer in Materials Science and Leader of the Biopolymer Group at mperial College, London.

Inverse lifetimes and the band structures of solids

from R.H. Williams

An accurate understanding of the behaviour of electrons in solids and the way that behaviour is related to the chemical composition and crystallographic structure is of considerable importance both fundamentally and technologically. The electrical resistivity of metals and insulators, and the behaviour of many solid state semiconductor devices, for example, depend on the detailed relationships between the energies and the momenta of electrons in those solids and accurate methods of probing the so-called 'band structures' have long been sought. Considerable progress has been achieved by studying such properties as the wavelength dependence of the optical absorption and reflectivity but during the past 10 years the technique of photoelectron spectroscopy has become one of the most promising and widely used. In the most commonly used form of the technique, electrons are emitted from the solid following irradiation by energetic photons and the energy distribution $N(E)$ of those electrons, which is clearly related to the occupied electron states, is measured. Quite recently the power of photoelectron spectroscopy has been vastly increased by the introduction of angle resolving techniques (see, for example, *Contemporary Physics*, **19**, 389; 1978) whereby a movable electron velocity analyser can probe $N(E)$ for electrons emitted in various directions. Assuming the momentum of the emitted electrons parallel to the surface to be conserved during the emission process then it becomes possible to establish this quantity inside the solid for the various electron states. Indeed for some materials, such as those which have a graphitic or layered structure, such measurements yield a good approximation of the full band structure which for the layered materials is largely two-dimensional in nature (see Larsen, Chiang & Smith *Phys. Rev. B* **15**, 3200; 1977). Interpretation of the experiments, however, may often be made complex due to the presence of the solid surface. The excited photoelectrons are strongly scattered by other electrons with the result that those which emerge without substantial loss of energy originate very close to the surface and may reflect the electronic structure of surface intrinsic or extrinsic (for example, due to absorbed gases) states. The short inelastic mean free path, or 'escape depth', of photoelectrons leads to a broadening of features in angle resolved photoelectron specroscopy and as pointed out by Pendry and Titterington (*Communications in Physics* **2**, 31; 1977) it should be possible from the peak widths in such experiments to determine not only the electron and hole lifetimes but also the electron mean free path and its variation with electron energy. This information is of considerable interest in the study of materials by electron spectroscopy.

Recently Eastman, Knapp, and Himpsel (*Phys. Rev. Lett.* **41**, 825; 1978; *Phys. Rev. B* **19**, 4952; 1979) and Thiry *et al.* (*Phys. Rev. Lett.* **43**, 82; 1979) have conducted inverse lifetime measurements of electrons and holes in copper single crystals using angle resolved photoelectron spectroscopy. At the same time the two groups have determined the electronic band structure of copper along well-specified crystallographic directions with unprecedented accuracy. Both groups used the power of angle-resolved photoemission and made use of the great advantages to be obtained by using synchrotron radiation as the light source (the synchrotron at the University of Wisconsin in the case of Eastman *et al.* and that at Orsay, France for Thiry *et al.*). Synchrotron radiation is a near ideal source for such studies as it is intense, highly polarised, and coupled to a suitable optical monochromator produces tunable photon energies over a large wavelength range. Both groups find that their spectra reflect occupied bulk electron states with momentum and energy being conserved in the emission process and by studying emission along a direction perpendicular to the crystal surface they were able to determine the full band structure along that direction. To map out the occupied electronic states it is necessary to have some knowledge of the states into

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R.H. Williams is Reader in Physics at the New University of Ulster, Coleraine, UK.

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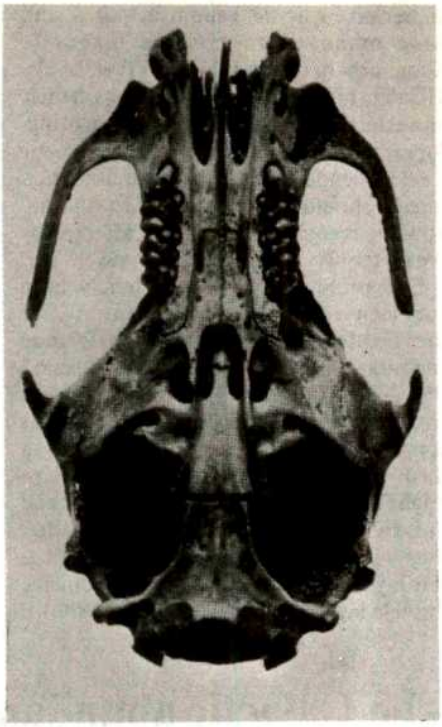
which the electrons are excited and Thiry *et al.* assume that excitation occurs into a free electron final state band. This procedure has previously been shown to give remarkably accurate results for the occupied states of some metals by Stohr *et al.* (*Phys. Rev. B* **17**, 587; 1978). Eastman's group makes use of the calculated electron states and both groups obtain very good agreement with theoretical predictions of the band structure of copper. Electron mean free paths determined from the experiments confirm the well known surface sensitivity of photoelectron spectroscopy particularly for excitation wavelengths below about 500 Å. Thiry *et al.* observe excited 3d hole lifetimes in copper which are in good agreement with the estimates of Pendry and Titterington, and further show the great importance of having a high angular resolution in the experiments.

These experiments again underline the power of angle-resolved photoemission coupled to a synchrotron radiation source as an accurate probe of electron states in solids. Studies similar to those described for copper, above, have also been conducted on nickel (Eastman, Himpsel & Knapp *Phys. Rev. Lett.* **40**, 1514; 1978) and the ferromagnetic exchange splitting accurately established. Coupled to other variations of the photoemission technique such as photoelectron diffraction (see *News and Views*, **279**, 755; 1979), which enable the locations of atoms near a solid surface to be established, an active and rewarding future in this area is certain, particularly as new dedicated synchrotron radiation sources such as the one at Daresbury in the United Kingdom become operational. These new methods will lead to a greater understanding of metals, alloys, semiconductors and insulators and will undoubtedly be important in probing areas such as surface reactions, oxidation of solids, and corrosion. □

The black rat in Britain

THE finding by archaeologists of remains of *Rattus rattus* in a filled-in Roman well in York in the north of England, suggests that the black rat was present in Britain several centuries earlier than had previously been supposed. The finds, which are discussed by James Rackham of the University of Durham in the latest issue of *Antiquity* (**53**, 112; 1979), lend support to the idea that epidemics of plague occurred in Britain well before the infamous Black Death of the Middle Ages, and may even have been common in Roman times.

Historians have assumed that the black rat — to be distinguished from its close relative the brown or Norway rat, *Rattus norvegicus* — was not introduced into Britain until the Norman period, when returning Crusader ships brought it from the Near East. The many contemporary records of 'plague' in Anglo-Saxon Britain in the 6th and 7th centuries AD have been either ignored or explained away as outbreaks of smallpox or other virulent diseases.



Black rat skull found in a Roman well in York.

There is no doubt that the high mortalities in the early centuries AD were often caused by a multitude of diseases, of which smallpox was certainly one. But it does not seem likely that Roman Britain would have completely escaped the plague epidemics that swept around the Mediterranean area and across Europe between the 1st and 6th centuries AD. The most serious of these pandemics — without doubt of bubonic plague — began in about AD 540 during Justinian's reign and spread from the Mediterranean up into central Gaul and Germany, and almost certainly would have reached the British Isles.

Plague usually spread from one coastal port to another because infected black rats would find their way on to and off ships. As there was extensive sea trading between Roman Britain and the rest of the Empire the introduction of rats into a British port could easily have happened. York was a thriving Roman administrative and trading centre. Like other large towns of the period, it was a port of call for supplies from many parts of Europe — indeed it is known to have had strong connections with Bordeaux and Rhineland. So it is not surprising that the black rat slipped into the town one day.

The rat remains recovered from the Roman well represent at least two individuals and include a well-preserved skull, a complete mandible with all its teeth, fragments of maxilla and premaxilla, and a cervical vertebra. The wall was timber-lined and was probably built in the late 2nd or early 3rd century and was then filled-in towards the end of the 4th century AD. During excavation careful checks were made to exclude the possibility that the rats had burrowed into the well after it had been sealed. This, and other evidence from Roman objects found in association with the rat remains and radiocarbon determinations suggest a 4th, or at the latest a 5th century date for the specimens.

These finds of the black rat in Roman Britain lend some support to John Wacher's controversial idea that plague led to the downfall of Romano-British towns in the 5th and 6th centuries AD at the time the Western Roman Empire was disintegrating. Wacher has suggested (*The Towns of Roman Britain*, Batsford 1974; *Roman Britain*, Dent 1978) that the depopulation of these towns was caused by epidemics striking an already threatened Romano-British culture. As economic decline set in, so the way was made easy for the conquering Anglo-Saxons and the start of the Dark Ages. S.B.

Quest for superfluid hydrogen

from P. V. E. McClintock

THE possibility of preparing monatomic hydrogen (H_1) at high densities seems to have come a stage closer with three papers published in recent issues of *Physical Review Letters*. Two separate research groups have simultaneously reported the first observations of magnetic resonance from H_1 held at liquid helium temperatures: Crampton, Greytak, Kleppner, Phillips, Smith and Weinrib of the Massachusetts Institute of Technology (*Phys. Rev. Lett.* **42**, 1039; 1979); and Hardy, Berlinsky and Whitehead of the University of British Columbia, Vancouver (*op. cit.*, 1042). On a slightly different tack, Guyer and Miller of the University of Massachusetts, Amherst, have calculated how H_1 may be expected to interact with a helium coating on the wall of its containing vessel (*op. cit.*, 1754).

The notion of monatomic hydrogen has been around for quite a long time. Hecht seems to have been the first to point out (*Physica* **25**, 1159; 1959) that H_1 at low temperatures and high densities might be expected to display quantum properties on a grand scale and, in particular, that it might undergo a superfluid transition rather like that of liquid ^{4}He. These possibilities rest on the observation that, although a pair of hydrogen atoms with oppositely directed electron spins attract each other and form a molecule of H_2, a pair of atoms with parallel electron spins (in a so-called triplet state) experience a repulsive interaction. This means that a gas of spin-aligned H_1 atoms could be cooled to very low temperatures, even at a high density, without ever condensing into a liquid, let alone solidifying, as do all other materials except helium (which stays liquid to absolute zero). The weak interaction forces between the atoms, coupled with the very low atomic mass, provide the ideal recipe for quantal behaviour: since the atomic mass is only a quarter that of ^{4}He, one may guess that the quantum properties of H_1 gas would be even more extreme than those of liquid helium.

Similar ideas can be applied to monatomic deuterim (D_1) and tritium (T_1), the heavier isotopes of hydrogen. Some interesting differences in their properties may be anticipated, however, because H_1 and T_1, each containing an even number of fundamental particles, are bosons, while D_1, with an odd number, is a fermion. Thus D_1 might perhaps be expected to behave rather like liquid ^{3}He, whereas H_1 and T_1 might each be expected to behave more in the manner of liquid ^{4}He. As gases, however, they would probably all have properties closer to those of ideal Fermi-Dirac and Bose-Einstein gases than the heliums. Such questions are of fundamental interest and importance for quantum mechanics and statistical mechanics, and this is a large part of the reason for the intense interest in seeing whether dense monatomic hydrogen can be made a reality on a macroscopic scale.

Although there has been a good deal of theoretical work on spin aligned hydrogen, with detailed predictions for many of its properties, relevant experiments have been somewhat thinner on the ground. The fundamental practical problem is, of course, to find a way of stabilising the H_1 gas against recombination to form H_2 molecules. The basic idea is to use a suitable container placed in an intense uniform magnetic field and held at a very low temperature. The field will induce a splitting in the ground state energy levels of the H_1 atom, depending on whether its electronic spin is pointing with or against the field; and it is hoped that the low temperature will ensure that the atom stays in the lowest level, that is, spin-aligned. It is believed that a field of 10 T and a temperautre of about 0.1 K might provide the necessary conditions for stable confinement of the H_1 gas. These conditions are realisable using existing cryogenic technology.

Before a full scale apparatus can be designed, however, it is necessary to obtain information about the properties of individual H_1 atoms at low temperatures and, in particular, about any mechanisms able to cause the spin flips which would rapidly lead to recombination to ordinary H_2. That is why the work reported from MIT and UBC is so important. Both groups have succeeded in conducting H_1 atoms down to experimental chambers immersed in liquid helium at 4.2 K and have managed to observe magnetic resonance between the hyperfine levels. This has now opened the way for further, more detailed, studies aimed at measuring, for example, the temperature and magnetic field dependences of the recombination rate. It should also be possible to find out how the recombination rate is affected by the material of the walls of the vessel.

One problem which had already been foreseen is that magnetic impurities inevitably present in the walls may encourage spin flips. A possible way round this difficulty would be to coat the walls with a layer of superfluid ^{4}He, which is completely non-magnetic. The question then arises as to whether the H_1 atoms might be able to enter the ^{4}He layer, and thus still be able to reach the walls; whether they will be trapped on the surface of the ^{4}He layer; or whether they will stay outside the ^{4}He layer altogether. Miller has shown (*Phys. Rev.* **B18**, 4730; 1978) that the H_1 is actually totally insoluble in liquid ^{4}He; Guyer and Miller now demonstrate that there will, in fact, be a shallow physiadsorbed state for H_1 just outside the ^{4}He layer, but that this state will not be heavily populated. They comment that ^{3}He impurities in the ^{4}He could give rise to difficulties since these, too, will congregate at the surface. This potential problem is easily circumvented however, because techniques have recently become available for the virtually complete removal of the 2 x 10^{-7} parts of ^{3}He found in commercial ^{4}He. So it looks very much as though a coating of isotopically pure superfluid ^{4}He will constitute an effective method for preventing wall-induced spin-flip processes.

Clearly, there is still a long way to go before superfluid hydrogen becomes a reality, but the present flourish of activity aimed in this general direction is most encouraging. □

P.V.E. McClintock is a Senior Lecturer in the Department of Physics at the University of Lancaster, UK.

The Galactic abundance gradient

from M.G. Edmunds

A CONSISTENT picture is at last beginning to emerge of the behaviour of the overall abundance of the elements, relative to hydrogen, as a function of position in our Galaxy. To a good approximation we can regard most elements heavier than helium as being produced in constant relative amounts during a single process — supernova nucleosynthesis — and the abundance of any one element is a fair indicator of the abundances of all the elements. There are of course a few exceptions, elements which are produced during quiescent stellar evolution such as nitrogen, but the majority are believed to come from supernovae. We can regard the Galaxy as a two-component system, made up of a disk of stars and gas, surrounded by a more-or-less spherical halo of old stars and globular star clusters. The pattern of the variation in abundance with distance from the Galactic centre in both disk and halo has become clearer with the publication of several papers over the past year.

K.A. James (*Astrophys. J. Suppl.* **39**, 135; 1979) has used narrow and wide band photometry to estimate metal abundance (anything heavier than helium) in a sample of giant stars and about 40 star clusters in the disk. Photometry can yield an abundance for a star because the strength of the absorption lines in the spectrum of the star's atmosphere is dependent on abundance, and the lines are not evenly distributed in wavelength. Thus a star with high abundances will emit less light in certain regions of its spectrum (particularly at short optical wavelengths), and more light in other regions, than will a metal-poor star. The differences are large enough to be detected by photometry of selected wavelength bands. The study of clusters is particularly useful because their distances can be estimated with reasonable accuracy, something that is very difficult to do for individual stars.

James finds there there is a distinct gradient of abundance decreasing outwards from the region of the Sun (10 kpc from the centre) to as far as he has measured at 14 kpc, although inwards towards the Galactic centre (from 10 kpc to 8 kpc) he claims to find little or no gradient. But his results are open to reinterpretation, and it may simply be that the classical notation for presenting abundances (as the logarithm of the quotient of the metal abundance relative to hydrogen in a star divided by the same thing for the Sun) may have obscured the actual trend. If James's data are replotted on a linear abundance scale, rather than a logarithmic one, then a simple straight line fits beautifully within the error bar in the plot of abundance against distance from the centre, both inside and outside the position of the Sun.

This therefore implies a simple linear abundance decrease in going from 8 to 14 kpc, a result which is in nice agreement with the indications of a linear abundance gradient in the interstellar material, deduced by observation of emission spectra of HII regions. There is much scatter in these derived nebular abundances, although this is probably mainly due to errors in observation and analysis, and it had originally been thought that this latter gradient was much steeper. But a reanalysis of the spectra assuming that temperature fluctuations in the nebulae are not important (as seems reasonable from recent theoretical modelling) gives a gradient in oxygen

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abundance in close agreement with this reinterpretation of James's result. (The reanalysis of the HII regions is discussed by Pagel in *Stars and Stellar Systems* ed. Westerlund, Reidel, 1979.)

If these gradients stay linear to the centre of the Galaxy, they imply a central enhancement over the solar neighbourhood by a factor of × 2.1 as judged by the 'metals' gradient, and a (negligibly different) factor × 2.5 as judged by the oxygen gradient. It may be dangerous to extrapolate so far, but interstellar obscuration makes optical observations in the disk difficult beyond 8 kpc. The extrapolation is anyway in encouraging agreement (within the probable errors) with a determination of heavy element abundances of about three times solar neighbourhood deduced by infrared observations of nebular lines from the Galactic centre (Aitken, Griffiths and Jones, *Mon. Not. R. astr. Soc.* **176**, 73p; 1976). Thus a linear abundance gradient in both stars and gas is indicated, with a factor of 2–3 decrease between the centre and the Sun. This conclusion will be of considerable value in improving estimates of the total gas content of the disk which rely on indirect derivations of H_2 abundance from millimetre-wavelength observations of co-existing carbon monoxide molecules. The interstellar CO abundance should depend linearly on the carbon abundance, given that the Galactic O/C ratio exceeds unity, and determinations have usually assumed a constant C/H ratio (fixed by observations near the Sun) throughout the disk. The H_2 content of the disk may thus be somewhat lower than previously thought.

For the halo, the situation is more uncertain. One of the principal components is the globular cluster system, and controversy has arisen over the existence or non-existence of a correlation between the overall metal abundance of a cluster and its distance from the Galactic centre. Undoubtedly there exists a great range in abundances for clusters at any given distance, but while Searle and Zinn (*Astrophys. J.* **225**, 357; 1978) claim — mainly on the basis of photometry — that there is no overall gradient, Harris and Canterna (*Astrophys. J. Lett.* **231**, L19; 1979) — partly on the basis of a compilation of high-dispersion spectral analyses — claim that there is. Comparison of abundances when stars have been analysed in different ways is always difficult, and resolution of the controversy may have to wait until a complete homogeneous set of analyses is available. The common ground between the two investigations is (1) there is a range in metal abundance of the clusters from solar to 1/100 of solar at all radii out to 10 kpc, and (2) there are no 'metal rich' clusters between 10 and 30 kpc, but a range between 1/10 and 1/100 of solar at any radius in this region. Beyond 30 kpc Searle and Zinn's sample is very small, but Harris and Canterna claim abundances less than or equal to only 1/100 solar out to 100 kpc. They include in their sample the dwarf outlying Draco and Ursa Major systems which are rather more open structures than globular clusters but almost undoubtedly part of the Galactic system. Searle and Zinn argue that, apart from the metal rich clusters which may have formed late in the early evolution of the Galaxy, a lack of gradient is apparent. This, together with the particular form of the spread of abundances at all radii, would indicate that the clusters essentially formed independently as stellar systems, lost gas, and then came together to form the halo. If Harris and Caterna's interpretation is preferred, in that the mean metal abundance at 50–100 kpc is lower than at 10-30 kpc and hence implying some gradient, then the assumption of independence would have to be modified so that the halo had some overall structure before and during formation of the clusters — rather than just aggregating together afterwards.

In the currently popular model, the Galaxy is presumed to have collapsed from a more dispersed state to its present form under the influence of self gravity — a model which, as Searle and Zinn point out, was first proposed by Kant as long ago as 1755. Abundance gradients can be produced easily in reasonably sophisticated computer simulations of the evolution of this model, but exactly what is the dominant process which causes the actual Galactic gradient is still not clear. Analytically, a linear gradient can be understood with the simplest possible model for synthesis resulting from converting gas into successive generations of stars, provided particular (but not unreasonable) radial distributions of gas and star mass are assumed (Pagel, *op. cit.*). But such a model does not give the observed unexpectedly small number of old stars in the disk with low abundances, and radial gas flows during the evolution of the Galaxy will probably have to be involved. The wide spread of abundances in the halo clusters certainly points to a fairly chaotic evolution there. Better knowledge of the spatial variation of element abundance in our own and nearby galaxies is certain to place useful constraints on our ideas of the formation and evolution of galaxies. □

M.G. Edmunds is a Lecturer in the Department of Applied Mathematics and Astronomy, University College, Cardiff.

Erratum

A line was accidentally omitted from the contribution of W.L. Fink and E.O. Wiley in a recent *News and Views* (*Nature* **280**, 542; 1979). In the fifth paragraph, the sentence beginning on the eighth line should read: "The lineage is broken up, naturally and non-arbitrarily, by the many speciation events which have occurred from the Devonian to the Recent."

Moas as rockhounds

from Ian Smalley

THOUSANDS of years before the first human set foot in New Zealand, the now extinct, flightless bird the moa, was roaming the countryside searching for semi-precious gemstones such as agate, opal, chalcedony, chert and jasper. It swallowed suitably sized pebbles of these materials and retained them in its gizzard to grind up its food. The birds carefully chose the hardest available stones and when they had worn smooth they spat them out — for latter-day geologists to find. B.W. Hayward of the New Zealand Geological Survey has reached this conclusion after studying the pebbles found on the sandy hills surrounding the Auckland University Field Club scientific station at Kawerua, on the west coast of Northland (*Tane* **20**, 159; 1978). The pebbles can only have come from the Waimamaku Valley some 5-10 km away; those examined had a mean maximum diameter of 28 mm and were highly polished and rather flattened in shape.

The birds seem to have been highly selective and to have concentrated on collecting and swallowing only the hardest of the local stones; these are the ones which, largely because of their hardness, fall into the semi-precious category. Before the arrival of man there must have been vast numbers of moas roaming New Zealand and they have left widespread and abundant deposits of used gizzard stones. In fact it seems that moas were common enough to have significantly affected the nature of the New Zealand vegetation, and the plant types seen today show the effects of long-term moa browsing (Greenwood & Atkinson *Proc. N.Z.Ecol.Soc.* **24**,21; 1977). The gizzard stones are virtually always varieties of quartz and exceptions have been observed in only two localities. The moas found in the well-known deposit at Pyramid Valley, North Canterbury used greywacke stones. This valley passes through Tertiary limestones and soft sandstones with greywacke exposed in the headwaters. There seems to be no nearby source of quartz and the next most resistant rock type available is greywacke; a similar situation exists for the stones from near Lake Tekapo.

At Kawerua one of the richest localities for finding pebbles is around the actively eroding Pukenuiorongo Bluffs. The Bluffs are 120 m above sea level and consist of a series of large washouts exposing a 40 m thick sequence of Pleistocene sands. This sequence probably accumulated on land as there is no evidence of water deposition

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Ian Smalley is with the Soil Bureau, Department of Scientific and Industrial Research, Lower Hutt, New Zealand.

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and although none of the sandstones preserves any laminations or crossbedding typical of dune deposits, they are probably wind blown accumulations having advanced inland from the coast. The stones were presumably coughed up by the moas in the sandy coastal areas and buried by the advance of the dune sand.

The pebbles collected from Pukenuiorongo Bluffs are generally flatter and more rounded and polished than most of the authenticated moa gizzard stones which are usually found in association with moa skeletons. This supports Hayward's hypothesis that the collected pebbles were supplied by live moas who coughed them up when their grinding efficiency was significantly reduced. Hayward also proposed that the moas sought actual rock types — even though the majority of the quartzose pebbles are gleaming white and easily distinguished the moas detected and swallowed numbers of brown, red and black pebbles of similar lithologies. It seems that the birds made their selection after a careful mineralogical assessment. □

Evidence for gluons?

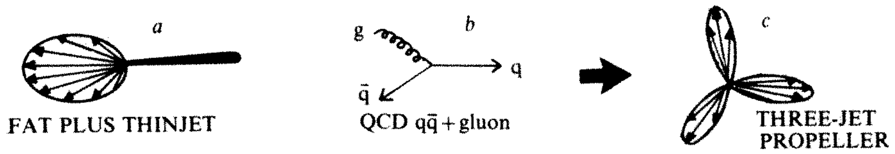
from Frank Close

THE latest results from PETRA, the Hamburg electron positron colliding ring, appear to add considerable weight to the quantum chromodynamic theory of the interquark force. Quarks are hypothesised to possess a new form of charge, known as colour, and interact with one another by exchanging coloured gluons (this process being somewhat analogous to the way that electrically charged objects mutually interact by exchanging photons). Just as the photon is the quantum of the electromagnetic force so are coloured gluons the quanta of the chromodynamic or interquark force. The theories of these forces are respectively quantum electrodynamics (QED) and quantum chromodynamics (QCD), the similarity in their names bearing witness to the similarity in their mathematical structures.

When electrons are annihilated by their antimatter (positrons) the energy is converted into light (this is well described by QED). This light lives for only a fraction of a second before being transformed into particles. Sometimes the light reconverts into leptons and antileptons (like the electron and positron). Alternatively the light may become a quark and an antiquark (q and $\bar{q}$). This process is conventionally denoted

$$e^+e^- \to \gamma \to q\bar{q}$$

So far everything is described by QED. In both cases electrical charges are being accelerated (positive and negative have been produced out of nothing) and QED predicts that quanta (photons) will be radiated by the produced charged particles (the lepton pair or the quark and antiquark). This is indeed the case experimentally and has been well understood for many years.



QCD for colour carriers is analogous to QED for charge carriers. Therefore it may be no surprise to learn that when colour charges are accelerated the theory predicts that gluons will be radiated. Quarks and antiquarks carry colour and so the real process is

$$e^+e^- \to \gamma \to (q + \text{photons} + \text{gluons}) + (\bar{q} + \text{photons} + \text{gluons})$$

From established QED we can calculate how much of the signal is due to the photon radiation. This then makes possible a study of the characteristics associated with gluon radiation from coloured quarks and comparison with the QCD predictions. There are three qualitative types of phenomena expected, one of which was only becoming apparent earlier this year (*Nature* **277**, 349; **280**, 450) and the third of which now appears to be showing up in the most recent Hamburg experiments.

(1) It is possible that the quark and antiquark may bind together and form a vector meson, like the J/ψ or Υ mesons, until such time as they annihilate one another thereby causing the meson to die. QCD predicts two things here. First, the probability for the quark and antiquark to annihilate decreases as the mass or energy of the $q\bar{q}$ bound state increases. This is apparently seen in tht the J/ψ (3.1GeV) and Υ (9.5GeV) have anomalously enhanced lifetimes compared to what would have been expected in the absence of QCD based on the known lifetime of the lighter ϕ(1.0GeV).

Second, it makes predictions as to the distribution in energy and space of the particles that are produced in the decay. The theory requires that the quark and antiquark annihilate, thereby producing three gluons. These gluons then transmute into pions and kaons that are detected by the experimental apparatus; three jets of particles should be detected bearing witness to the memory of the three gluons.

The J/ψ at 3GeV is too light to allow a good test of this prediction. Studies of the more massive Υ early in 1979 began to provide data supporting the three-jet prediction (*News and Views* **277**, 349; **280**, 450). The best tests of this prediction may have to wait until (if?) a yet heavier vector meson (θ) is discovered, a bound state of the hypothesised sixth quark (t and $\bar{t}$). Such a meson, (if it exists) appears to be too massive to be produced at the present generation of electron-positron machines.

(2) The energy of the electron-positron annihilation might not be suitable for a vector meson to be simply produced. In this case the q,$\bar{q}$ and associated glue are radiated off away from one another.

QCD predicts that, at high energies, the most likely configurations are where the quark and antiquark carry off most of the energy and the glue is either very soft or highly collimated along the $q\bar{q}$ axis. As a consequence two jets of particles will be seen in the detectors.

Such 'two-jet events' have been known to exist for about 5 years. QCD predicts that as the electron-positron annihilation energies increase, so these two-jet events will become increasingly collimated and pencil-like. Data at the highest energies indeed support this.

(3) Although these jetlike — pencil — events should dominate, there is a chance that the quark (or antiquark) radiates a hard gluon which carries off momentum and deflects the quark (antiquark) from its path (Fig 1*a*). If resolution is poor this will show up as a thin-jet plus fat-jet event, like a squash or tennis racket (Fig. 1*a*). Evidence for such events was reported by the TASSO collaboration at the CERN conference in June.

The latest results from PETRA are obtained with it operating at the highest energy of 32GeV. The events are dominantly two-jet in character but a significant number of planar tennis racket type were seen. Some of these do appear to be resolvable into a genuine three-jet structure (Fig 1*c*) (known as propeller or Mercedes events — the German laboratory inspired by the manufacturers symbol). These latest results were announced at a conference at Fermilab, Chicago during the final week of August.

So it appears that, at least qualitatively, the behaviour of high energy electron-positron annihilation does mimic the expectations of QCD, the theory of the interquark force. Theorists and experimentalists suspect that these three-jet events are due to a quark radiating a gluon before the detected particles are created Figure 1*b* shows the $q\bar{q}$ gluon state, which then becomes particles like pions and kaons by an unknown mechanism. However, there is much quantitative work still to be done by experimentatists, and theorists will be trying to find more precise tests. With more work at the highest energies, PETRA seems well set to be able to perform a significant test of the theory. □

F.E. Close is with the Theory Division, SRC Rutherford Laboratory.

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review article

Structure and evolution of the Moon

Stuart Ross Taylor

Research School of Earth Sciences, Australian National University, Canberra, Australia

The scientific investigation of the samples returned by the Apollo and Luna missions, together with geophysical and photogeological data, has provided a reasonably complete understanding of the structure, composition and evolution of the Moon, and has placed many constraints on theories of its origin.

THE tenth anniversary of the first of the manned Apollo missions (20 July 1969) to the Moon provides an appropriate opportunity to review the current state of our knowledge of the structure, composition, evolution and origin of the Moon. Much of our scientific understanding stems from the samples obtained during the Apollo missions. Coupled with the various geophysical and photogeological information also obtained, and reinforced by the unmanned missions to Mars, Mercury and Venus, the result has been the development of the science of planetology. Insights into the origin of the Solar System, the composition of the solar nebula, and the relation of the Earth to the other members of the inner parts of the Solar System have all been obtained. The current state of lunar and planetary science is illustrated by over 50,000 pages of literature written since 1969. The latest additions to this mountain of data are the 1,426 pages of abstracts of papers for the tenth Lunar Science Conference held in Houston, Texas, in March 1979. Within the limits of this survey, it is not possible to reference adequately all this material, or to do justice to many of the important scientific results. Some critical and interesting areas (soils, track studies, rare gases, and meteorite flux) have had to be excluded because of space limitations.

Internal structure

Models for the internal structure of the Moon down to depths of 1,000 km have now reached a consensus (Fig. 1). The lunar crust, which is less dense than, and chemically distinct from the interior, varies in thickness from 60 km to 100 km. These values are constrained partly by density[1] (crustal density = 2.96 g cm^{-3}), but also by some seismic evidence for the base of the crust[2,3]. The mare basalts, although prominent visually, constitute less than 1% of the crustal volume (0.1% of lunar volume). The highland crustal thickness is 60 km at the Apollo 12 site and 75 km at the Apollo 16 site. A 20-km discontinuity within the crust does not reflect the base of the mare basalts, but rather the depth of fracturing or of closure of cracks produced by the large collisions, thus representing the change from fractured to competent rock[2,3].

The upper mantle or lithosphere extends from the base of the crust (60–100 km) to 400–500 km. The average P wave velocity (V_p) is 7.7 km s^{-1} and the average S wave velocity (V_s) 4.45 km s^{-1}, with negative velocity gradients. There is some inconclusive evidence for lateral heterogeneity[4]. A minor discontinuity exists[2,3], most probably located at a depth of 480 km, with a change to lower seismic wave velocities (V_p 7.6 km s^{-1}; V_s 4.2 km s^{-1}) down to depths of about 1,000 km. Deep within this zone (800–1,000 km depth) occur most of the moonquakes. A few (so called high frequency teleseismic events) occur at depths of about 100 km, most probably associated with deep fractures surrounding the ringed basins[5]. The crust itself is aseismic. Below 1,110 km, the structure becomes less certain. P and S waves become attenuated (V_p ~5.0 km s^{-1}; V_s 2.5 km s^{-1}), consistent with the presence of a small amount (~1%) of melt. There is a major discontinuity at about 1,110 km, with a very sharp decrease in S wave velocities[2,3].

A lunar core? The possible existence of a lunar core has been much debated but the evidence for it remains inconclusive. The seismic data restrict the core radius to 170–360 km (less than 2% of lunar volume)[6]. The lunar coefficient of moment of inertia[7] constraint ($1/MR^2 = 0.391 \pm 0.002$) requires a density increase in the deep interior, in addition to the low density crust. Although such a constraint may be satisfied by a core, it is also satisfied by a density increase at the 480 km discontinuity to a more iron-rich interior[2,3].

The restrictions on core size to about 2% of the lunar volume make such a core of little geochemical significance, for example as a sink for the siderophile elements. The presence of a core implies complete lunar differentiation. Most of the evidence (discussed in later sections) favours initial melting of half or two-thirds of the Moon rather than a completely molten body.

Whether such a small core could have generated a magnetic field, via conventional dynamo action, remains uncertain, as do the origin and intensity of the ancient lunar magnetic field. The surprising initial observation that the returned lunar samples possessed a stable natural remanent magnetisation (NMR), which indicated the former presence of a now vanished magnetic field, has continued to baffle investigators. Considerable efforts have been made to determine the palaeointensity of this field, and there is general agreement that fields up to 1 Oe were required on the lunar surface to account for the NMR[8–10]. Some evidence exists for a decay in field strength over about two orders of magnitude between 4,000 and 3,000 Myr but the evidence, particularly from the breccias, is still a matter of controversy[9,10]. This is leading to a concentration of effort on the mare basalts, which possess a much simpler history than the complex breccias[10]. Debate continues about the local or Moon-wide nature of the field[11]. Resolution of this problem may have to await a future lunar orbiter. The locally varying magnetic anomalies observed by the orbiting subsatellites during the Apollo 16 mission definitely correlate with ejecta blankets from the ringed basins and older large craters[12]. The youngest identifiable area yielding such an anomaly is the feature Reiner gamma, dated stratigraphically at 2,900 Myr.

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The origins proposed for the lunar magnetic field encompass a large number of processes, which fall within the following categories: (1) the field is random due to localised near-surface origin[13]; (2) external uniform fields; (3) internal dipole field[4]. No resolution seems possible at present between these alternatives[10]. Note that attempts to relate the lunar field to the presumed interplanetary field responsible for the fossil magnetism observed in meteorites may be misleading. Among other caveats, the fields responsible for the magnetic effects in meteorites were in existence about 1,000 Myr earlier than those responsible for the lunar magnetism[14].

The lunar highlands

Crustal densities, based on orbital γ-ray data for iron, are consistent with models of uniform density and variable crustal thickness (Airy-type model) rather than the reverse (Pratt model[15]). Contrary to earlier interpretations[16], the Apennine Mountains, rising 7 km about Mare Imbrium, may be isostatically uncompensated, supported by the lunar crust and lithosphere for 3,900 Myr[17]. Coupled with the mascon data, this provides a constraint on lunar thermal models and on the strength of the lunar interior. Partial melting in the interior, which produced the mare basalts as late as 3,200 (or 2,600) Myr, did not weaken the upper lithosphere sufficiently to allow isostatic compensation. Younger large lunar craters (for example, Copernicus) show negative gravity anomalies (that is, net mass deficiency) but older craters such as Humboldt do not[18]. The possibility that the older craters are isostatically compensated is not consistent with the presence of the uncompensated Apennines. Possible explanations include the reduction of porosity in the subcrater structure by shaking during the Imbrium and Orientale basin forming collisions at 3,900 Myr, or the intrusion of mare basalt as sills and dykes into the fractured subsurface structure.

The large ringed basins (with up to five concentric mountain rings as for Orientale) owe their origin to collisions of asteroids or planetesimals. The energy release during the Imbrium collision has been estimated[19] at about 3×10^{33} erg (corresponding to the impact of a 100-km diameter asteroid of density 3 g cm^{-3} travelling at 16 km s^{-1}), about two orders of magnitude less than the energy required to disrupt the Moon[19]. Opinion has been divided between an origin of the outer rings by the initial cratering episode or by later slumping along concentric faults. The present view based on cratering experiments[20] is that the rings formed, like nested craters, during the impact[21–23].

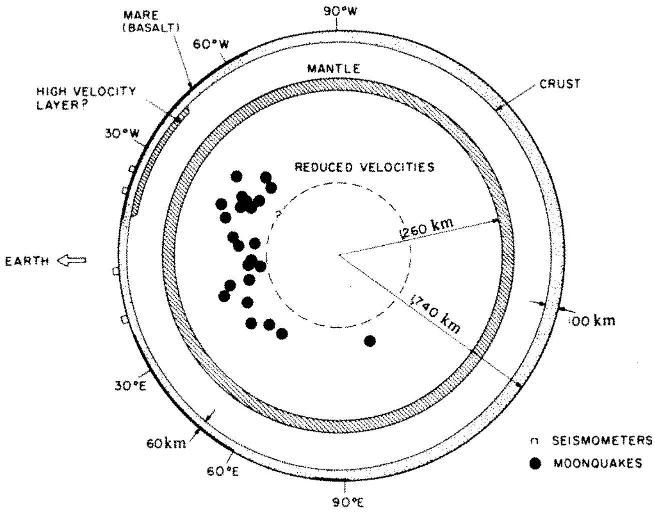

Fig. 1 Internal structure of the Moon[2,3], consisting of a 60–100-km thick crust overlying a two-layered mantle, or lithosphere, with a transition zone between 400 and 480 km. Epicentres of typically observed moonquakes are shown. The inner region, within the dashed circle, shows a sharp decrease in P and S wave velocities. (Courtesy N. E. Goins.)

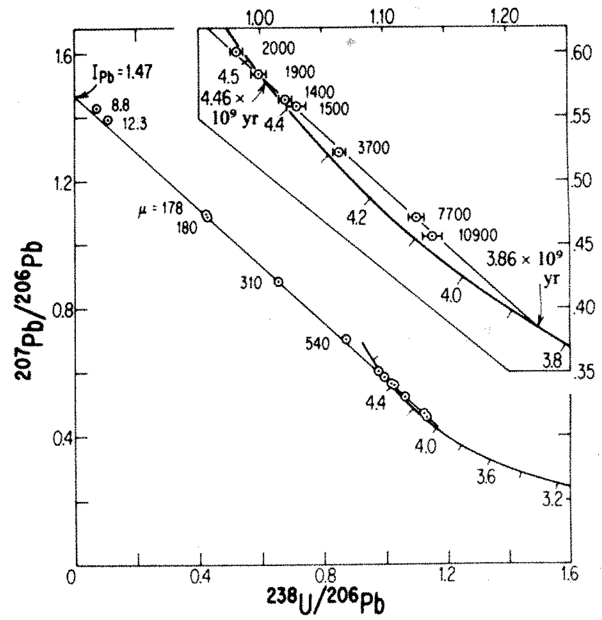

Fig. 2 U–Pb evolution diagram for highland breccias[28]. Inset shows data points near concordia curve with error bars. The measured value of $\mu = {}^{238}\text{U}/{}^{204}\text{Pb}$ is given for each sample. Of 13 data points, 10 lie precisely on a line intersecting concordia curve at 4,460 Myr and 3,860 Myr. The upper intersection represents the time of early lunar differentiation and crust formation. The lower intersection corresponds to a time of intense bombardment of the Moon (terminal lunar cataclysm). Samples whose data points do not plot precisely on the line may have impact ages slightly different from 3,860 Myr. The cataclysm affected almost all lunar highland rocks, causing severe U/Pb fractionation in the breccias. This is reflected by the linear distribution of the data points on the graph and the strong correlation of μ values with position of the data points. (Courtesy F. Oberli and G. J. Wasserburg.)

The depth to which the impacts which produced the giant ringed basins have excavated and overturned (and thus sampled) the crust remains uncertain. Estimates have ranged from minimum values[24] of 8–27 km to depths exceeding the thickness of the lunar crust[25]. An upper limit is provided by the lack of material collected from beneath the feldspathic crust. The dunite (72415) which is sometimes so described has a mineralogy indicative of shallow, rather than deep, origin[26]. It may have originated either as a cumulate, crystallising in a pool of impact melt, or, more probably, on account of its old age (4,600 Myr), from liquid trapped in the feldspathic crust, or possibly in a frozen primordial crust. It is thus of doubtful use as an index of the composition of the lunar mantle.

The mechanics of formation of the giant ringed basins are critical for the interpretation of the samples returned from the lunar highlands. Sampling of the 'light plains' units in the highlands (at Apollo 16) demolished the theory that they were of volcanic origin. They are due to the widespread transport of debris across the lunar surface, during the excavation of the giant ringed basins. However, the primary basin ejecta does not blanket the pre-existing surface for more than a short distance from the basin rim. The secondary projectiles flying out from the explosion plough up the surface and create their own local ejecta. The result is that the light plains units are mainly composed of locally derived debris. Thus, the ejecta blanket from the Imbrium collision, which was sampled at the Fra Mauro site during the Apollo 14 mission, although intensely brecciated, does not show the effects of the more than 200 kbar shock pressure which existed at the centre of the Imbrium basin during impact[27]. The orbital γ-ray and X-ray fluorescence data show much variation across the highlands, consistent with local chemical variability and with the above model.

The date of the formation of the lunar highland crust has been refined as a result of lead isotope investigations (Fig. 2). These isotopic data show two ages, one at 4,460 Myr, which is interpreted as the age of formation, and a second at 3,860 Myr consistent with the age of the Imbrium collision[28]. These new dates place the crystallisation of the highland crust a little earlier than the 4,400 Myr previously accepted. As the date for the condensation of the Solar System is usually given as 4,570 Myr[29], accretion of the Moon followed by melting and crystallisation of at least half the lunar volume, was accomplished very quickly.

The overall composition of the lunar highland surface is given in Table 1. It is highly fractionated with respect to either primitive solar nebula compositions or to the bulk composition of the Moon (Table 1). Although early workers were so impressed by this refractory alumina-rich composition as to suggest a late, heterogeneous accretion episode to account for it, all geochemical and petrological evidence now links the highland crust to the rest of the Moon. For example, the similarity in the ratios of volatile/refractory elements (for example, K/Ba, K/Zr, K/U) in all returned lunar samples[30] indissolubly links the highlands and the mare samples derived from the lunar interior.

The question of whether a feldspathic crust would float over a crystallising 'magma ocean' was resolved by experimental results[31] which demonstrated that such flotation would occur in the anhydrous lunar environment in liquids parental to the highland crust. No report of water on the Moon, even at the level of parts per 10^9 (p.p.b.), has been substantiated. This contrasts with the terrestrial situation, where the presence of small quantities of water causes the feldspar to sink. This, among other factors, invalidates the case for an early anorthositic crust on the Earth[32,33].

Detailed models for the crystallisation of the magma ocean[34–36] indicate that such processes were complex, probably involving the formation of 'rockbergs' (analogous to icebergs), cycles of assimilation and mixing, and trapping of interstitial liquids to produce the complex suite of crustal rocks. It is probable that an extensive bombardment continued throughout the formation of the crust and for the next 500–600 Myr, culminating in the terminal cataclysm at about 3,900 Myr to which the Imbrium and Orientale basins bear witness. The complexity of the resulting rock types has led to a literature too extensive to review here (see, for example, ref. 37).

In addition to the feldspathic (anorthositic) and primitive Mg-rich components in the lunar crust, a third, known as KREEP, is pervasive. Its origin has been widely debated since its discovery at the Fra Mauro site during the Apollo 14 mission (following traces reported from the Apollo 12 landing site). The continuing bombardment which pulverised the crust has destroyed most of the evidence. One view considers that the KREEP basalts are derived from flows representing pre-mare volcanism[27]. The absence of identifiable rocks (except for those crystallising from impact melts) makes this view, based on photogeological interpretation, less likely than the other extreme, which relates the origin of KREEP to the crystallisation of the magma ocean. In this model, the extremely high concentrations of such components as K, rare earth elements (REE), P, Ba, Th, U and Zr are derived from the residual melt following crystallisation of the magma ocean[38]. This model is reinforced by the Sm–Nd systematics[39] which indicate a uniform origin for KREEP at the different landing sites, and by the parallel REE patterns independent of total REE abundance which typify many highlands samples[40].

The mare basalts

Such models for the origin of the crust are related to the question of the source of the dark mare basalts, which have been remarked on in most mythologies. They cover 17% of the lunar surface, although only rarely (in the centres of ringed basins) exceeding thicknesses of 1 km (refs 41, 42). Their concentration on the Earth-facing hemisphere, where the crust is thinner (Fig. 1) is probably a consequence of non-uniform crustal thickness[43]. The mare basalts are derived by partial melting from depths of 100–400 km. Hence, they provide, in principle, information about the composition of the deep interior and so the nature of their source regions has been keenly debated.

Several recent reviews have appeared[44–47] which delineate nearly 20 types ranging from high titanium to very low titanium (VLT) basalts. Most of these types cannot be related simply by partial melting or by fractional crystallisation from a single parent magma, but demand differing source compositions. The nearly ubiquitous depletion in Eu demonstrates prior removal from the source regions of plagioclase. Isotopic systematics (Rb–Sr and Sm–Nd) (see, for example, ref. 48) indicate crystallisation of the source regions at or before 4,400 Myr, hundreds of millions of years before their eruption. Dated flows extend

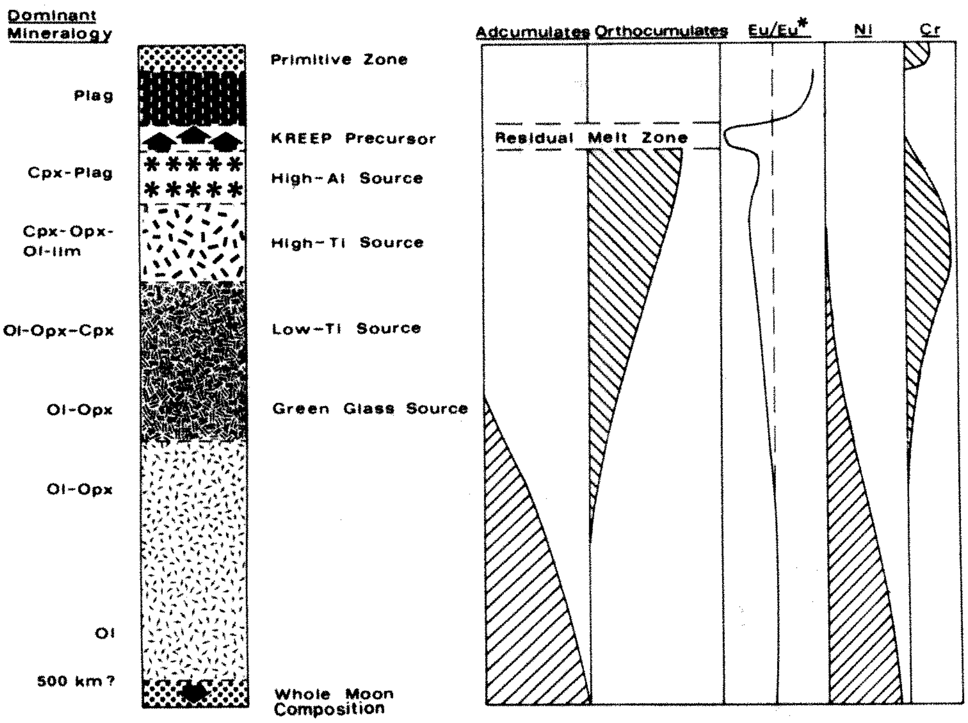

Fig. 3 Schematic diagram to illustrate formation of a mineralogically zoned source region for the lunar mare basalts, during crystallisation of a 500-km deep magma ocean. Adcumulus growth (that is, little trapped pore liquid) predominates at depth, with orthocumulus growth (incorporation of trapped interstitial liquids) occurring at higher levels. The Eu anomaly (Eu/Eu*) increases to a maximum negative value in the KREEP precursor zone, and is positive in the floating plagioclase crust. Nickel is concentrated in olivine-rich zones in the deep interior. Chromium, possibly mainly divalent[61,62], is concentrated in the source regions of the mare basalts. (Adapted from ref. 57.)

from 3,900 Myr to 3,160 Myr[40]. Mare volcanism probably began earlier, before the final cataclysmic collisions, both from photogeological studies[48] and the presence of basalt clasts in Apollo 14 breccias[40]. The style of eruption is analogous to terrestrial plateau or flood basalts. The combination of low viscosity and high flow rates[50] produced the characteristic smooth (±150 m) surfaces of the mare basalts[51]. The impacts which formed the large basins, although producing some local melt[52], did not initiate the floods of basalt from the lunar interior. These were erupted up to hundreds of millions of years after the great collisions. Among the new types of basalts[47,53–55] described recently, the most significant are the Apollo 17 VLT basalts[55]. These display the high V and Cr and low Ni contents typical of most mare basalts, but in addition have heavy REE (HREE)-enriched patterns, rather than the flat or depleted patterns common to other species. The REE patterns, which include a negative Eu anomaly, preclude derivation either from a primitive interior, or by assimilation of crustal or cumulate material[55].

When contemplating the whole spectrum of basalt types, derivation from a cumulate source region remains the most promising model. The crystallisation of the magma ocean produces a layered series of differing mineralogy, ranging from olivine and orthopyroxene at the base to clinopyroxene–ilmenite cumulates at shallower levels (Fig. 3). Trapping of interstitial liquids, convective overturn, magma mixing and other processes[35] will greatly complicate the simplified models originally envisaged[38] but the overall concept accounts for both the isotopic systematics and trace element abundances. The total absence of water and other volatile components means that small-scale heterogeneities in the lunar interior will remain frozen in. A consequence is that mare basalt compositions (even the most primitive such as Apollo 15 green glass[56] or Apollo 17 VLT basalts[55]) are derived from fractionated source regions and do not provide direct information on the primordial lunar composition.

Much progress has been made in mapping mare basalts from the orbital chemical data[58] and by remote sensing techniques[59]. The youngest mare basalts, identified in Western Oceanus Procellarum by photogeological techniques, are about 2,600 Myr old[60]. After this time, no lavas reached the surface. The melting of mare basalts in the interior was fuelled by trapped K, U and Th, although most of the lunar budget for these radioactive elements was concentrated in the crust by the early differentiation.

A frozen surface. The cessation of mare basalt flooding at 2,600 Myr caused the last discernible changes to the lunar surface (Fig. 4) except for the production of younger impact craters, such as Copernicus, Kepler, Aristarchus and Tycho. The last major structural change to the lunar surface occurred at 3,900 Myr with the production of the 900-km diameter Orientale ringed basin, with an energy release[19] estimated at 1.4×10^{33} erg. Since that event, the lunar surface shows no evidence of major tectonic activity, expansion or contraction (except for minor adjustments around the edges of the maria), enabling limits of ±1 km to be applied to the change in lunar radius over the past 4,000 Myr[63]. This lack of change rules out current claims for massive increases in Earth radius[64] and restricts the rate of change of G ($-\delta G/G\ \mathrm{yr}^{-1}$) to $\leq 5\times10^{-11}$ in constant mass cosmologies, and to $\leq 1.5\times10^{-10}$ in variable mass cosmologies[65].

Lunar composition

The overall composition of the Moon is of particular interest because of its possible relationship to the Earth. The highly fractionated nature of the lunar samples to which we have access place constraints on all models. The heat flow[66] measured at the Apollo 15 and 17 sites is consistent, for a steady-state model, with bulk lunar uranium contents in the range 35–46 p.p.b. A non-steady-state solution could reduce these values by about 20% (ref. 67). A bulk lunar U content of about 40 p.p.b. is estimated from geochemical balance considerations[68]. From correlations among refractory elements (Ca/Al/Ti/U ratios), from K/U systematics (K/U = 2,500) and from the lunar density (3.34 g cm^{-3}) which constrains the Fe content, it is possible to arrive at compositions which satisfy the density, moment of inertia and heat flow constraints. An average of such compositions is given in Table 1. Melting of the outer half or two-thirds of the Moon of such composition gives rise to the feldspathic lunar crust[35] as well as a zoned interior capable of producing the mare basalts by partial melting. The depth of initial lunar melting[57,63] is most probably 300–500 km. If the seismic discontinuity[2,3] at 480 km is identified with the base of the melted zone, then the seismic velocity decrease (Fig. 1) and the coefficient of moment of inertia value, are consistent with a change to a primitive unfractionated iron-rich interior. The lower portions of the melted zone will be dominated by Mg-rich olivine and orthopyroxene so that a sharp break in seismic velocities is expected. In this model, partial melting in the deep

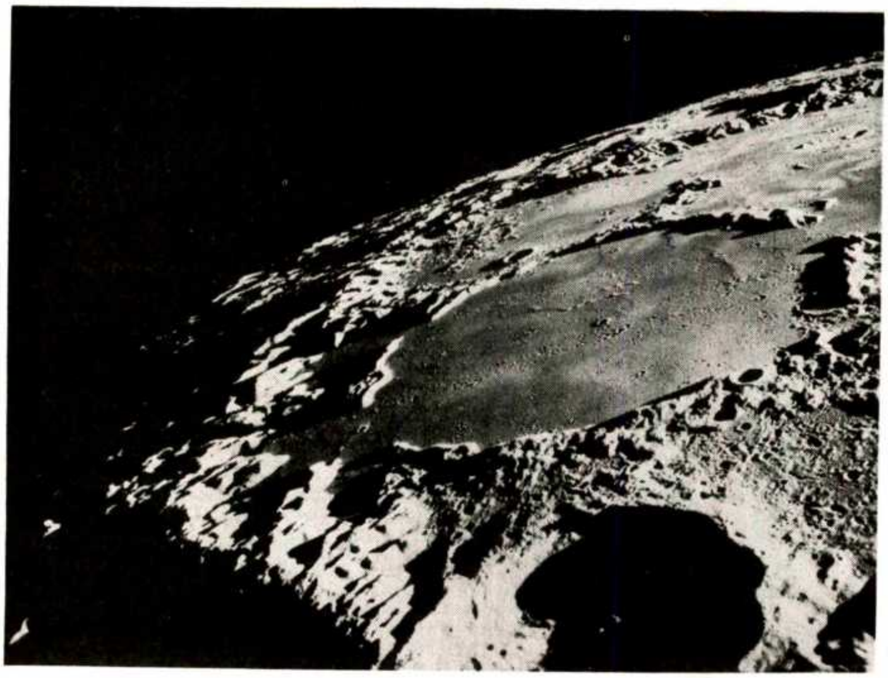

Fig. 4 Farside lunar maria and highlands. The large circular crater, filled with mare basalt, is Thomson (112 km diameter) in the northeast sector of Mare Ingenii (370 km diameter, 34° S, 164°). The large crater in the right foreground is Zelinskiy (54 km diameter). The stratigraphic sequence, from oldest to youngest, is (1) formation of highland crust, (2) excavation of Ingenii basin, (3) formation of Thomson crater, (4) formation of Zelinskiy, (5) flooding of Ingenii basin and Thomson crater with mare basalt, (6) production of small craters on mare surface including a probable chain of secondary craters (NASA AS15-87-11724).

interior is caused by the primitive component of K, U and Th.

Tektites. The disproving of the hypothesis of the lunar origin of tektites must be counted among the minor successes of the Apollo missions. Recent attempts to revive the hypothesis that they are derived from young lunar volcanoes[69] encounters similar problems (for example, one to two orders of magnitude age discrepancy, chemical and isotopic distinctions, lack of observed Tertiary lunar volcanoes) to those which ruled out an origin by meteorite splash from the Moon[70].

Lunar origins

The earlier debate over homogeneous versus heterogeneous accretion of the Moon was resolved in favour of the former hypothesis[30,40]. Homogeneous accretion of a refractory-rich, and volatile and siderophile element-poor Moon, was followed by extensive melting. The evolution of the Moon is thus well understood, but the pre-accretion scenarios remain indistinct. The three principal pre-Apollo hypotheses all encountered difficulties when confronted with the new data.

Little progress has been made with simple versions of the double planet theory but the oxygen isotope data, perhaps the most significant single cosmochemical observation in the past few years, indicate a close association between the Earth and the Moon[73]. The depletion in the refractory siderophile elements forbids direct accretion from the solar nebula, so that one or more stages of element fractionation are needed between condensation of the nebula and accretion of the Moon. The location of these events is obscure.

Capture of an already formed Moon has a *deus ex machina* flavour, and has been deemed 'horrendously improbable' (ref. 74). Such an origin, in a different part of the solar nebula, could be predicted to produce some distinct chemical or isotopic signature. In this context, the oxygen isotope data[73] may have dealt a fatal blow to the capture hypothesis, by requiring formation of the Earth and Moon in close association. Nevertheless, the presence of fractionated meteorites (enstatite achrondrites and aubrites) with similar ^{16}O enrichments, and the close similarity of the howardites and eucrites to the terrestrial values must engender caution, as more efficient mixing may occur in planetary size bodies. Acquisition of oxygen isotope data from other planets should be accorded a high priority, along with age dating, for future sample return missions.

Fission from the Earth has been extensively debated, because in principle, an evaluation of the theory can be achieved by comparison of lunar compositions with that of the terrestrial mantle. This task is in fact complex, partly due to our lack of understanding of the lateral and vertical variations in the composition of the terrestrial mantle (for an extensive recent review see ref. 75). The siderophile element abundances are particularly crucial, but the reasons for the high abundances of such elements (for example, Ni) in the upper mantle of the Earth remain obscure. It is clear that the terrestrial upper mantle has not been in equilibrium with the core of the Earth[72]. Possibly, the high abundances of elements such as Ni are derived from a post-accretion meteoritic bombardment of the Earth[76–78] analogous to such early events on the Moon, Mars and Mercury.

Whatever the cause of the terrestrial siderophile element abundance patterns, the concentrations of refractory siderophile elements Os, Ir, Ni, Pd and Re are enriched by large factors in the Earth compared with the Moon[79]. Au, Sb and Ge are depleted by factors of about 100 in the Moon while the very volatile elements (Bi, Tl, In) show rather uniform depletions of about 50 in the Moon[79]. The alkali elements K, Rb and Cs similarly show depletions by factors of about 2 between the Moon and the mantle of the Earth (Table 1). All these comparisons are complicated by the differing evolutionary histories of the Earth's mantle and the Moon. Simple comparisons, for example, of basalts from both bodies, encounter this problem. Elements such as K and U whose bulk composition can be estimated from a variety of techniques (K–Ar systematics, K/Rb/Sr, Ca/Al/U ratios and heat-flow values) are useful in this context[71]. Terrestrial bulk U values of 15–20 p.p.b. are a factor of 2 lower than the lunar abundances (Table 1). Similar constraints apply to the refractory elements Ca, Al and Ti. The Moon also contains more Fe and less Mg than the Earth's mantle (Table 1).

Table 1 Composition of the lunar highlands, the bulk Moon and the Earth's mantle

	1	2	3	4
SiO_2%	45	42	46.1	45
TiO_2%	0.56	0.4	0.2	0.16
Al_2O_3%	24.6	8.0	4.3	3.3
FeO%	6.6	12	8.2	8.0
MgO%	8.6	31	37.6	40
CaO%	14.2	6.0	3.1	2.6
Na_2O%	0.45	0.1	0.4	0.2
K (p.p.m.)	600	80–100	250	150
U (p.p.b.)	240	30–40	—	15
Th (p.p.b.)	900	120–150	—	60
K/U	2,500	2,500	—	10,000

1, Lunar highlands surface[40]; 2, bulk Moon[71]; 3, Earth's mantle[72]; 4, Earth's mantle[71] (NiO = 0.26, Cr_2O_3 = 0.44). p.p.b. = parts per 10^9.

Thus, the weight of the chemical evidence seems to indicate that the terrestrial mantle and the Moon differ substantially in composition for many elements, refractory, volatile and siderophile. The similarity in chemistry between the eucrites and the lunar basalts indicates the existence of similar processes elsewhere in the inner Solar System[80,81]. The recent demonstration[82] that the siderophile and volatile element contents of the shergottite meteorites mimic those of terrestrial basalts weakens the case for fission. Thus, the initial attraction of the fission hypothesis (the density similarity between the Moon and the terrestrial mantle) is diminished. Accordingly, very complex scenarios have to be devised in order to derive the Moon from the mantle of the Earth[83], or for forming the Moon in close association with the Earth[75,84,85]. The formation of the Moon from a ring of differentiated debris in terrestrial orbit[86,87] during the accretion of the Earth accords most closely with the oxygen isotopic evidence[73].

Epilogue

Could our insights into the composition, age and evolution of the Moon have been obtained from unmanned missions? The answer is no. Such missions would have returned lunar soils. The formation of these soils was not well understood before the Apollo missions. The unravelling of the age, isotopic and chemical evidence which they contain was only possible because individual components (the hand-collected whole rock samples) of these complex mixtures were also available. Our experience gained from the Apollo missions enables us to understand and extract chemical and age information from such samples[47], so that now the scientific examination of an unmanned sample collected and returned from Mars or Mercury could be approached with confidence.

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articles

Suspended sediment sources identified by magnetic measurements

D. E. Walling & M. R. Peart
Department of Geography, University of Exeter, UK
F. Oldfield
Department of Geography, University of Liverpool, UK
R. Thompson
Department of Geophysics, University of Edinburgh, UK

A procedure for identifying the main source of the suspended sediment load of a stream by simple, cheap, rapid and nondestructive magnetic measurements is applicable to samples taken during the routine sampling of flood events permitting general characterisation of the load and more detailed investigation of variations in sediment source both during and between individual flood events.

INFORMATION on the sources of the suspended sediment loads of rivers, and more particularly the relative importance of sheet and rill erosion and channel scour, has many potential uses in studies of sediment transport and sediment yield. For example, it could provide the geomorphologist with a means of elucidating patterns and rates of erosion and afford a means of highlighting potential problems of nonpoint pollution connected with sediment-associated nutrients and contaminants derived from agricultural land. Existing techniques for determining the relative magnitude of these sources have involved detailed documentation of source area erosion[1] and comparison of measured sediment loads with estimates of sediment yield from sheet erosion derived using a soil loss equation coupled with a sediment delivery term[2]. The first approach involves many operational problems and is essentially impossible for anything except small drainage basins, whilst the latter introduces many uncertainties related to the accuracy of the techniques used to estimate sheet erosion and sediment delivery. Furthermore, both approaches provide only long-term aggregate values and do not permit the study and comparison of individual events. Scope clearly exists for alternative procedures and the use of the properties of the sediment load itself as a means of 'fingerprinting' source areas has considerable promise.

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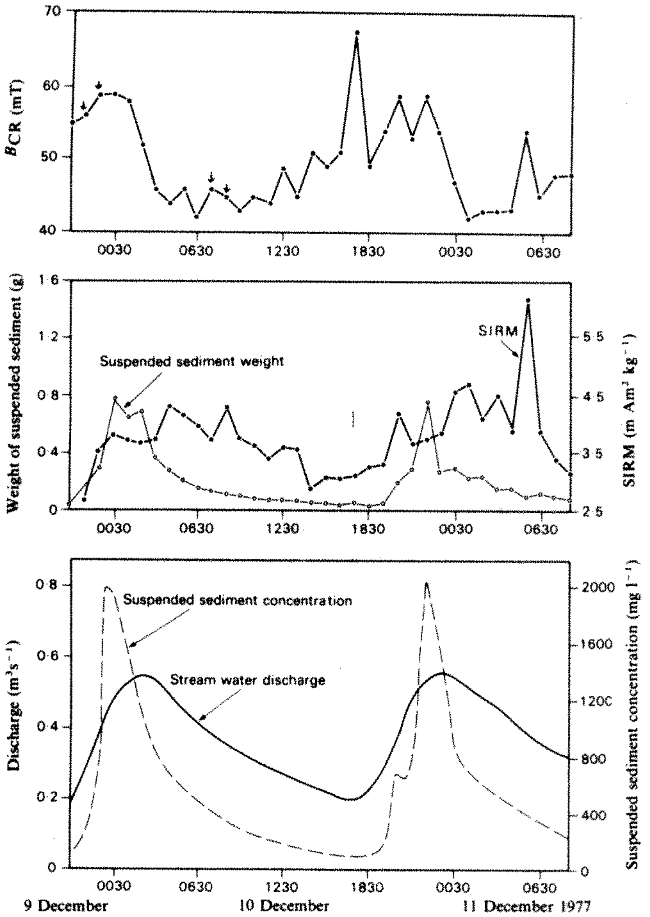

Fig. 1 Variations in B_{CR}, SIRM and suspended sediment concentration measured during a double peaked hydrograph on the Jackmoor Brook. The four samples indexed on the B_{CR} plot are those used to produce the coercivity profiles presented in Fig. 2.

Measurements at Jackmoor Brook

Oldfield *et al.*[3] have previously given preliminary results of an attempt to identify the dominant source of the suspended sediment yield from the Jackmoor Brook basin, an instrumented catchment in south-west England, by comparing the magnetic properties of material from potential source areas within the catchment with those of samples of sediment in transit. Their results showed that bulk magnetic susceptibility (χ), saturation isothermal remanent magnetisation (SIRM), SIRM/χ ratios and coercivity of SIRM (B_{CR}) profiles (Table 1) provide an exceptionally rapid and sensitive basis for differentiating undisturbed woodland topsoil, arable soil, and streambank erosion scars cut into the unmodified Permian bedrock. The processes controlling the transformation of iron compounds which make this type of differentiation possible are poorly understood[4] but in this case they give rise to an alteration of the fine grained weakly ferromagnetic haematite cement in the unweathered parent material to a ferrimagnetic form during soil development. Fire often causes this transformation, although other less dramatic pedogenic processes may also be responsible. The resulting cubic oxide has been identified as either maghemite[5-7] or magnetite[8]. Most evidence suggests that it is impure as a result of isomorphic substitution of iron by other soil cations such as aluminium.

All the magnetic properties previously noted are in some measure diagnostic of this transformation and they permit crude magnetic 'fingerprinting' of different types of sediment source. Oldfield *et al.*[3] showed that the magnetic parameters of the four bulk suspended sediment samples measured were closely comparable to those of cultivated topsoil, but that they differed significantly in several ways from either undisturbed woodland soil or unaltered parent material (Table 1). This suggested that the bulk of the suspended sediment transported by the stream was generated by splash erosion and surface runoff from the extensive cultivated areas of the catchment rather than by channel scour and by erosion of bankside scars. Minor differences in B_{CR} and in SIRM/χ ratios between the individual suspended sediment samples seemed to be related to the discharge conditions prevailing at the time of sampling and the characteristics of the associated storm runoff event. These differences prompted us to look in more detail at variations in the magnetic properties of the suspended sediment transported by this stream both during and between individual storm events.

Transport of suspended sediment

In the previous study, samples of suspended sediment in transit used for magnetic determinations had been obtained by centrifuging bulk samples of stream water (>20 l) and drying the residual solids. More detailed work on individual storm runoff events necessitated a larger number of samples and in view of the operational problems involved in collecting and transporting numerous bulk samples of stream water and the time consuming nature of the laboratory centrifugation procedure, an alternative means of assembling sediment samples for magnetic measurements was sought. It was found that similar measurements could be undertaken on the residue retained on a glass fibre filter circle after filtration of a relatively small volume of river water (~500 ml). The measurements were unaffected by the presence of the filter medium carrying the sediment. Furthermore the samples collected by automatic pump sampling equipment installed at the outflow gauging station of the Jackmoor Brook catchment could be used for this purpose. This equipment, developed from an earlier design[9], collects discrete samples at regular six-hourly intervals and is also triggered to collect samples at hourly intervals when the water stage rises above a preset threshold during storm events. The samples have

Table 1 Summary of selected magnetic properties of bulk suspended sediment samples and potential sediment sources from the Jackmoor Brook catchment

Material	Property χ* (mm³ kg⁻¹)	SIRM† (mA m² kg⁻¹)	SIRM/χ‡ (kA m⁻¹)	B_{CR}§ (mT)	S‖
Woodland topsoil	>2.5	>10	≈4	>20	1
Cultivated topsoil	0.2–2	1–10	5–7	24–41	0.28–0.8
Poorly drained and gleyed soils	0.06–0.4	0.5–3.5	≈10	≈30	≈0.4
Parent material	<0.1	1–2	>10	200–400	−0.8–−0.6
Suspended sediment	0.25–0.75	2.5–9	≈10	37–60	0.06–0.4

* Specific magnetic susceptibility. A measure of the magnetisability of a specimen. Measured in an alternating field of about 0.1 mT peak strength with a frequency of 10 kHz. Mainly reflects the concentration of ferrimagnetic minerals in a specimen.

† Saturation isothermal remanent magnetisation. The magnetisation of a specimen remaining after its removal from a strong (1 T) magnetic field. Mainly reflects the concentration of magnetic minerals in a specimen, but also influenced by the size and shape of magnetic grains.

‡ This ratio reflects the type, size and shape of the magnetic minerals present. For example, it will be decreased by an increasing proportion of ultrafine (superparamagnetic) magnetic grains.

§ Coercivity of remanence. A measure of the stability of the SIRM, being the reverse field required to reduce the remanent magnetisation to zero after saturation in a forward field. Reflects the magnetic mineral type, size or shape, not influenced by concentration.

‖ This ratio of the remanence grown in a back field of 100 mT to the SIRM, mainly depends on the ratio of haematite to magnetite minerals in the specimen. A high S value indicates a relatively greater proportion of magnetite in the sample.

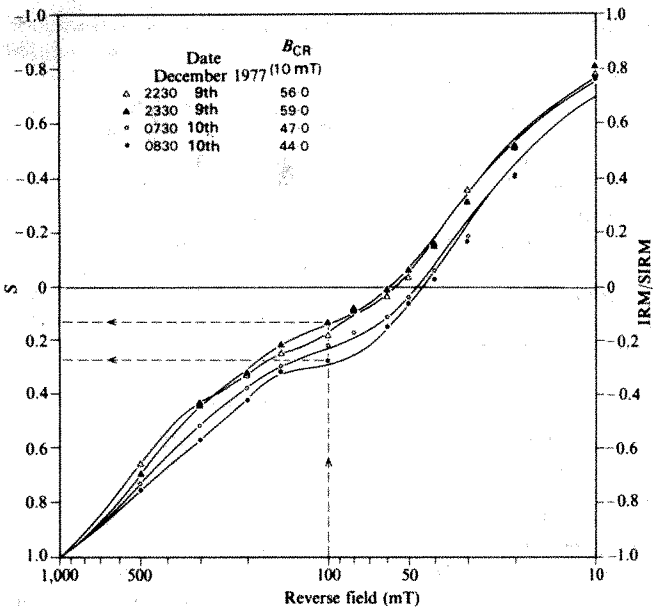

Fig. 2 Coercivity profiles obtained from filter paper residues associated with four samples collected during the storm shown on Fig. 1. Values of S for these samples range from 0.135 to 0.271 and are consistent with the dominance of a cultivated top soil source (Table 1).

been found to be representative of the ambient suspended sediment concentrations at the moment of sampling and may be related to the continuous records of water discharge and suspended sediment concentration (turbidity) available for the gauging site. Samples were therefore collected at frequent intervals during a number of storm events occurring between December 1977 and March 1978.

Samples collected by the automatic sampling equipment were returned to the laboratory for vacuum filtration through pre-weighed glass fibre filter circles (Whatman GFC). Subsequent drying and weighing provided information on the weight of sediment carried by individual filter circles and these were finally moistened, folded, and inserted into polystyrene sample holders with flexible airtight lids.

The SIRM of all resulting samples was determined by placing each one in a 'saturating field' of 1 T and measuring the induced remanent magnetisation (IRM) on a computerised fluxgate magnetometer[10]. The noise level of this instrument was two orders of magnitude below the signal from the least magnetic of the samples measured. The signal from the holder and filter medium was minimised by washing every container and lid in distilled water, by handling samples with clean hands and by keeping both empty and full holders in clean sealed polythene bags. Measurements giving values less than four times the mean signal from empty holders containing clean filter circles were rejected. The consistency of the remaining results encourages confidence in measurements made on samples with dry weights as low as 0.02 g (that is well over 90% of the sample analysed). The SIRM measurements, which are specific to unit mass and therefore independent of sample weight, have been completed for over 160 samples covering discharge conditions ranging from 0.05 to 1.0 $m^3 s^{-1}$ and sediment concentrations ranging from 50 to 2,500 mg l^{-1}. Suites of hourly samples spanning five distinct flood events have been analysed.

For the first suite of samples covering the period 2130 h on 9 December 1977 to 0830 h on 11 December 1977, coercivity of SIRM profiles were also obtained for all of the 36 samples. Figure 1 plots the hourly values of SIRM, B_{CR}, and sediment weight along with the record of discharge and suspended sediment concentration for the event. Coercivity profiles were calculated from measurements of remanence in 10–12 reverse fields of increasing strength. Figure 2 shows representative coercivity profiles drawn automatically from the measurements using a smoothing cubic spline function.

For subsequent sample suites, coercivity measurements were made only on extreme samples for which the SIRM values fell close to the outside limits spanned by the first suite. It was also possible to determine magnetic susceptibility for a proportion of the samples with values above the level of repeatable measurement (1×10^{-7} $m^3 kg^{-1}$) and calculate their SIRM/χ ratios.

The data on the magnetic properties of suspended sediment transported by Jackmoor Brook, assembled using the filter paper technique described above enable us to evaluate variations in these properties both within and between individual events in terms of the sediment sources and erosion processes involved.

Discussion

Figure 1 illustrates the variations in B_{CR} and SIRM measured during a double peaked hydrograph on the Jackmoor Brook. The variations exhibited are typical of most storm hydrographs and indicate a clear tendency for B_{CR} values to decrease during times of increased runoff and sediment concentration and for SIRM values to increase. The range of values shown are very

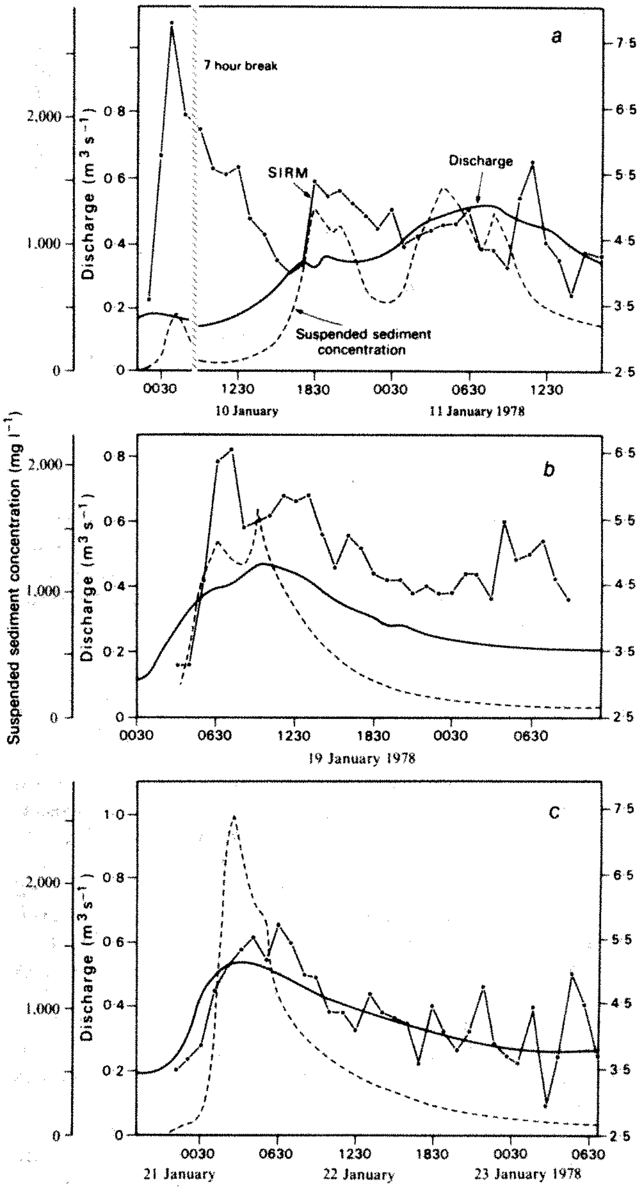

Fig. 3 Variations in SIRM and suspended sediment concentration measured during three storm runoff events on the Jackmoor Brook.

similar to those obtained in the earlier study which suggested that cultivated soils provide the major source of sediment within this catchment (Table 1). Decreasing B_{CR} values and increasing SIRM values indicate a tendency for such sources to become more dominant at the time of the hydrograph peak, because arable soils are marked by higher SIRM and lower B_{CR} values than are the channel-side erosion scars (Table 1). This pattern of variation during the storm hydrograph is therefore consistent with a situation where maximum sediment yields from cultivated areas would coincide with maximum rainfall intensities, maximum surface runoff rates, a maximum area contributing to surface runoff, and therefore the hydrograph peak. Although there is a tendency for the maximum sediment concentrations to precede the discharge peaks in Fig. 1, the maximum SIRM and minimum B_{CR} values occur after the hydrograph peak. This lag effect is again consistent with the sediment sources and process mechanisms that could be postulated for the catchment. Some channel scour must occur and the maximum contribution from this source would be expected to occur during the rising stage of the hydrograph and at the hydrograph peak in response to control by sediment availability and the erosive energy of the streamflow. Many workers[11] have noted that sediment contributions from bank erosion are closely controlled by the loosening of sediment particles during preceding dry periods and that the supply from this source is rapidly depleted. The magnetic properties of the suspended sediment would therefore be most influenced by bank contributions during the earlier part of the streamflow hydrograph and this would delay the occurrence of maximum SIRM and minimum B_{CR} values until after the peak flow had passed. It is tempting to attribute the slight reversal in the trend of increasing SIRM values associated with both hydrograph peaks (0030–0230 and 2030–2330 h on 12 December) to a maximum contribution from channel scour at this time but a more detailed sampling programme would be necessary to confirm the precise nature of this reversal. In addition, the higher SIRM values exhibited by the second hydrograph peak are readily explicable in terms of the depletion of channel sources during the first peak and therefore a lesser contribution from channel scour.

Comparison of a number of individual sediment yield events is also facilitated by the ease with which data from filter paper measurements may be collected and Fig. 3 presents the values of SIRM measured during three storm events which succeeded that documented in Fig. 1. Considerable contrasts in levels of SIRM are apparent between these events but the contrasts are again consistent with a simple process-based conceptual model of the variations in the relative importance of slope and channel sources to the sediment budget of this catchment. The full range of SIRM values (2.8–8 mA m^2 kg^{-1}) indicate that agricultural soils provide the dominant source on all occasions (see Table 1) but relatively low values may be interpreted in terms of proportionally greater contributions from channel scour. The event shown in Fig. 1 was the first major hydrograph of the winter period and might be expected to reflect a relatively high contribution from this source and therefore lower SIRM values. Sediment particles loosened during the preceding dry period would provide a ready supply to channel scour. This source would, however, be rapidly depleted and the higher SIRM values obtained for the two subsequent events (Fig. 3*a* and *b*) may be explained both in terms of lack of sufficient time for the supply to be restored and lower discharges which are less effective for channel scour.

The hydrograph which occurred on 22 January 1978 (Fig. 3*c*) exhibits lower SIRM values than the preceding two events and this feature may be ascribed to the higher discharges involved and therefore to increased channel scour. Higher discharges involve both higher velocities and shear stresses and also submerge greater areas of the channel margin. However, although the discharge peak for this event is of similar magnitude to that of the hydrograph depicted in Fig. 1, the amount of channel scour could be expected to be less than occurred during the first flood event of the winter in view of the depletion effect discussed above and this is confirmed by a comparison of the SIRM values. The very high values of SIRM (up to 8 mA m^2 kg^{-1}) associated with the early part of the storm which occurred on 10 January 1978 (Fig. 3*a*) are also worthy of comment. In this instance the increase in sediment concentration is not related to a significant increase in discharge and it is unlikely that channel scour could be invoked as the source. A localised input from a field source seems more likely and this is consistent with the high SIRM values which may be viewed as being the result of a lack of dilution by sediment from channel sources. Low values of B_{CR} were also evident during this event.

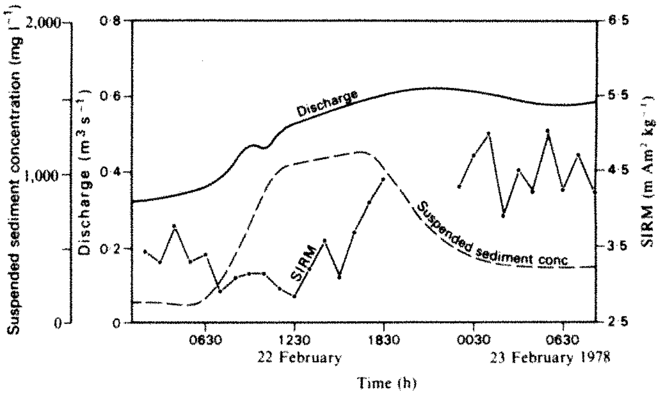

Fig. 4 Variations in SIRM and suspended sediment concentration measured during a snowmelt runoff event on the Jackmoor Brook.

A final example of variation in the magnetic properties of sediment between individual events is given by Fig. 4. This shows a hydrograph which was unusual in having been produced by snowmelt rather than rainfall. The relatively low suspended sediment concentrations may be explained by the lack of rainfall energy, an important component of splash erosion. SIRM values are also somewhat low and this is consistent with a situation unconducive to slope erosion and where channel scour would be of increased relative importance compared to other events.

Conclusions

Measurements of the magnetic properties of suspended sediment in transit can provide a general indication of the relative importance of slope and channel sources to the sediment yield of a catchment, and more specific information on the relative contributions of the individual sources during a storm event and on contrasts in the pattern of sediment generation between particular events. The use of filter paper residues for magnetic determinations provides a simple, effective means of collecting the detailed data required for studies of inter- and intra-storm variations. More work is required on sediment enrichment ratios, on the relationship between magnetic properties and the particle size and organic matter content of suspended sediment, and within catchments draining other rock types, if the potential for using magnetic determinations in studies of sediment sources is to be fully realised.

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Fundamental difference between the molecular interactions of agonists and antagonists with the β-adrenergic receptor

Gregory A. Weiland, Kenneth P. Minneman & Perry B. Molinoff

Department of Pharmacology, University of Colorado Medical Center, 4200 East Ninth Avenue, Denver, Colorado 80262

Antagonist binding to the β-adrenergic receptor is largely entropy driven, with only a small enthalpy component. The binding of agonists, on the other hand, is associated with a large decrease in enthalpy which permits a highly unfavourable decrease in entropy. The thermodynamic differences between the binding of agonists and antagonists may provide new insights into the molecular basis for hormone stimulation of adenylate cyclase activity.

THE binding of compounds to the β-adrenergic receptor has been examined in great detail with the use of high-affinity radiolabelled β-adrenergic ligands such as ^{125}I-iodohydroxybenzylpindolol (IHYP)[1-3]. These studies have increased our understanding of the first step in the activation of adenylate cyclase by catecholamines, but have provided little insight into how binding effects an increase in enzyme activity. It is likely that agonists induce a change in the conformation of the receptor and that this altered form of the receptor interacts, directly or indirectly, with the enzyme to cause activation. Thus, an agonist-induced increase in the apparent Stokes radius of the digitonin-solubilised β-adrenergic receptor has been reported[4] and it has been shown that agonists cause an increase in the sensitivity of the β-adrenergic receptor to *N*-ethylmaleimide[5]. These results are consistent with the idea that conformational changes occur, but they provide relatively little information about the molecular mechanisms involved.

Differential effects of agonists and antagonists on receptor conformation should be readily apparent in the thermodynamics of the binding reaction. In fact, agonist but not antagonist affinities for β-adrenergic receptors increase at lower temperatures[6,7]. There is also indirect physiological evidence that agonist affinities are higher at lower temperatures, whereas antagonist affinities are unaffected by changes in temperature[8-11]. We report here that the thermodynamics of agonist and antagonist interactions with the β-adrenergic receptor of turkey erythrocytes are substantially different. This difference apparently reflects specific agonist-induced changes in receptor conformation.

Effect of temperature on ligand binding to the β-adrenergic receptor

The turkey erythrocyte membrane is a widely used model for the β-adrenergic receptor/adenylate cyclase system[1,4-6]. It is a convenient system in which to examine the effects of temperature, as the association and dissociation of IHYP binding to turkey erythrocyte β-adrenergic receptors occur 6–10 times faster than with other β-adrenergic receptors[12]. Incubation times required to reach equilibrium are therefore much shorter in the turkey erythrocyte membrane. The effect of temperature on the inhibition of IHYP binding by (−)propranolol and (−)isoprenaline are shown in Fig. 1. The displacement curve of the agonist isoprenaline shifts to the left at lower temperatures, reflecting an increase in affinity, whereas there is little change in the apparent affinity of propranolol. A similar increase in the apparent affinity of isoprenaline with decreasing temperature was also observed in studies of isoprenaline-stimulated adenylate cyclase activity (Fig. 2).

The changes in affinity observed are not a reflection of changes in *p*H as the K_D values of propranolol and isoprenaline did not change significantly between *p*H 6.8 and 7.8. The changes were also not an artefact of the longer incubation time used at lower temperatures, as the affinity of the receptor for isoprenaline measured at 37 °C after 5 h at 1 °C was the same as its affinity determined after only the 37 °C incubation. The K_D for isoprenaline at 1 °C was also unchanged by prior incubation for 15 min at 37 °C. The observed changes in affinity are therefore reversible and apparently a function of temperature alone. Full agonists displayed the largest increases in affinity with a lowering of temperature whereas the affinities of antagonists were essentially unchanged (Table 1). The effect of temperature on partial agonists was less than that on full agonists.

Thermodynamic analysis of the temperature dependence of binding

The standard Gibbs free energy change (ΔG°) of binding (association) was calculated from the equation

$$\Delta G^\circ = -RT \ln K_A \tag{1}$$

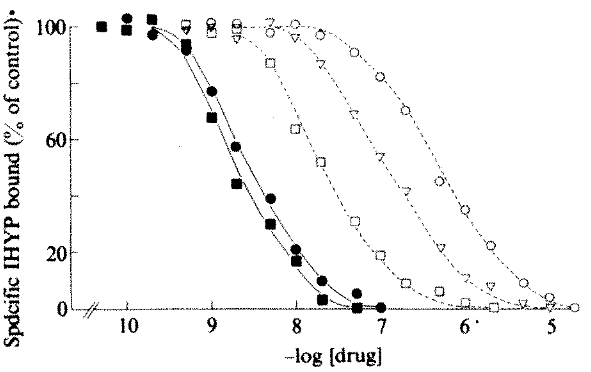

Fig. 1 Effect of temperature on the inhibition of specifically bound IHYP by (−)isoprenaline and (−)propranolol. Turkey erythrocyte membranes were prepared as described by Øye and Sutherland[33] and stored at −70 °C in 0.145 M NaCl containing 10 mM Tris-Cl (*p*H 7.4) and 1 mM EDTA at a protein concentration of 40 mg ml^{-1}. Immediately before use the membranes were thawed and diluted 100-fold in ice-cold isotonic saline containing 20 mM Tris-Cl (*p*H 7.5). Following centrifugation at 20,000*g* for 10 min at 4 °C, the membranes were resuspended in buffered saline to a protein concentration of 0.1 mg ml^{-1}. ^{125}I-iodohydroxybenzylpindolol (IHYP, 2.2 Ci μmol^{-1}) was prepared and purified as previously described[2,3]. Turkey erythrocyte membranes (10 μg protein) were incubated with IHYP (45,000–55,000 c.p.m.) and competing ligand ((−)isoprenaline: ○, ▽, □; (−)propranolol: ●, ■) in 0.25 ml of 0.09 M NaCl containing 1.1 mM ascorbic acid and 12 mM Tris-Cl (*p*H 7.2 at 24 °C) for 15 min at 37° (○, ●). 2.5 h at 10 °C (▽), and 5 h at 1 °C (□, ■). Bound IHYP was determined by filtration through glass fibre filters as previously described[34]. GTP (100 μM) had no effect on either agonist or antagonist binding at 1 °C or 37 °C. Specific binding was defined as the amount of IHYP bound in the absence of competing ligand minus the amount bound in the presence of 30 μM (−)isoprenaline. Specific binding routinely represented 90% of the total IHYP bound. Each point represents the mean of three experiments done in duplicate.

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Table 1 Equilibrium dissociation constants of β-adrenergic ligands at 37 °C and 1 °C

	K_D(nM) 37 °C	K_D(nM) 1 °C	$K_D(37\,°C)/K_D(1\,°C)$
Agonists			
(−)isoprenaline	254±18	11±2.0	23.3
(−)noradrenaline	2,680±280	48±1.5	55.5
(−)adrenaline	5,230±960	326±95	16.0
Partial agonists			
Soterenol	1,620±70	305±51	5.3
Fenoterol	3,160±250	868±56	3.6
Salmefamol	7,460±460	1,130±80	6.6
Metaproterenol	16,800±2,200	1,670±510	10.1
Terbutaline	43,900±7,200	18,200±1,300	2.4
Antagonists			
(−)propranolol	1.6±0.21	0.59±0.032	2.6
MK-950	2.1±0.18	2.5±1.06	0.84
IPS-339	2.2±0.43	2.3±0.49	0.95
Pindolol	4.5±0.12	1.5±0.39	2.95
Zinterol	372±29	196±19	1.92
Metoprolol	1,300±180	1,130±20	1.15
Sotatol	1,660±90	1,050±150	1.58
H 35/25	2,510±440	1,700±100	1.48
Butoxamine	3,760±440	2,990±84	1.26
OPC 2009	4,810±250	3,970±330	1.21
Atenolol	5,300±90	2,530±510	2.09
Practolol	5,640±520	12,600±4,400	0.45

IC_{50} values were determined from Hill plots of the inhibition of specific IHYP binding at 37 °C and 1 °C (see Fig. 1). K_D values were calculated from the IC_{50} values by the method of Cheng and Prusoff[36] using the equation: $K_D = IC_{50}/(1 + L/K_L)$, where L is the concentration of free IHYP and K_L the equilibrium dissociation constant for IHYP determined by Scatchard analysis. Hill coefficients for the binding of ligands did not differ significantly from 1.0. K_L was 42 pM at 37 °C and 20 pM at 1 °C. The number of IHYP binding sites (B_{max}) was 372± 10 fmol per mg protein at 37 °C and 375±10 fmol per mg protein at 1 °C. The results shown are expressed as the mean±s.e.m. for 3–10 determinations done in duplicate.

where R is the gas constant (1.99 cal mol^{-1} deg^{-1}), T is the temperature in degrees Kelvin, and K_A is the equilibrium association constant ($1/K_D$). Changes in the standard Gibbs free energy reflect changes in enthalpy ($\Delta H°$) as well as changes in entropy ($\Delta S°$) as

$$\Delta G° = \Delta H° - T\Delta S° \qquad (2)$$

The enthalpy change of the binding reaction was determined using the integrated van't Hoff equation

$$\ln K_A = -\Delta H°/RT + \Delta S°/R \qquad (3)$$

The slope of a plot of $\ln K_A$ against $1/T$ is $-\Delta H°/R$. Once $\Delta G°$ and $\Delta H°$ had been determined, the standard entropy change of the reaction ($\Delta S°$) could be calculated from equation (2).

Van't Hoff plots for the binding reaction of five representative β-adrenergic ligands are shown in Fig. 3. The plots are linear over the temperature range studied, showing that the enthalpy change is independent of temperature. Both the enthalpy and the entropy changes of agonist binding are substantially different from those of the antagonists, despite the overlap in the changes in Gibbs free energy (Table 2). The most striking finding is that binding of full agonists results in a decrease in entropy. This decrease is thermodynamically unfavourable and it is only because of the large decreases in enthalpy that binding can occur. Thus, the binding of agonists is enthalpy driven, with marked net decreases in entropy, whereas the binding of antagonists seems to be almost completely entropy driven.

An excellent correlation was observed when the decreases in enthalpy and the efficacies of full and partial agonists were compared (Fig. 4*A*). Antagonists have zero efficacy and their binding is associated with an increase in entropy ranging from +13 to +42 entropy units (cal deg^{-1} mol^{-1}) (Fig. 4*B*). Full agonists had $\Delta S°$ values of less than −12 entropy units, and the binding of partial agonists had intermediate entropy values.

Molecular interpretations of the thermodynamics of binding

The entropy-driven binding of antagonists to the β-receptor is thermodynamically similar to many other protein-binding reactions where information transfer is not involved. Thus, for the interaction of ovalbumin with rabbit anti-ovalbumin antibody, $\Delta H° = 0$ kcal mol^{-1}, and $\Delta S° = +20$ entropy units (EU) (ref. 12), and for the binding of organic ions to bovine serum abumin, $\Delta H° = -2$ kcal mol^{-1} and $\Delta S° = +14.5$ to $+17.1$ EU (ref. 13). These reactions are probably driven by the increases in entropy which result when water molecules, ordered around the ligand and the binding site, are displaced[14]. In view of the relatively non-polar nature of the β-adrenergic receptor antagonists, it is likely that the binding is largely hydrophobic although other forces, including ionic interactions, hydrogen bonding and Van der Waals interactions, may also be involved. However, net enthalpy changes would not be expected because dissolution of energetically similar bonds with water would necessarily occur. In any case, these types of interaction are also entropy driven[15,16].

The thermodynamics of agonist–receptor interactions are substantially different from those of antagonist binding and other passive ligand–protein reactions. Note, however, that the thermodynamic parameters measured are net changes resulting from the interaction of a ligand and protein. These thermodynamic changes can also reflect induced conformational changes in the receptor or in adjoining molecules. As discussed above, binding reactions in general have a large positive entropy component. It is reasonable to suggest that occupation of the receptor by agonists also results in an increase in entropy. The net decrease in entropy observed on agonist binding may reflect

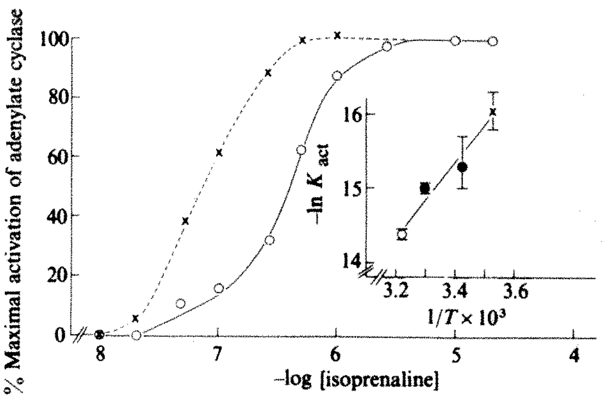

Fig. 2 Effect of temperature on the concentration dependence of (−)isoprenaline activation of adenylate cyclase. Adenylate cyclase activity was determined by measuring the conversion of [α-^{32}P]ATP to ^{32}P-labelled cyclic AMP and isolating the product by a modification of the method of Salomon *et al.*[35] as previously described[34]. The reaction was carried out in a final volume of 0.2 ml including turkey erythrocyte membranes (1–3 mg protein), 50 mM Tris-maleate (*p*H 7.5 at 24 °C), 5 mM cyclic AMP, 0.75 mM isobutylmethylxanthine, 5 mM $MgCl_2$, 0.5 mM EGTA, 0.25 mM ATP, 0.1 mg ml^{-1} creatine kinase, 10 mM creatine phosphate and 30 μM GTP. Reactions were run for 10 min at 37 °C (○) and for 60 min at 10 °C (×). Each point represents the mean of three experiments done in duplicate. Basal activities (in pmol cyclic AMP formed per min per mg protein) were: 0.55± 0.045 at 37 °C and 0.060±0.0024 at 10 °C. Maximal per cent stimulation by isoprenaline decreased with decreasing temperature. Isoprenaline stimulated adenylate cyclase 7.8-fold at 37 °C, 3.2-fold at 30 °C, 2.2-fold at 20 °C and 1.3-fold at 10 °C. Adenylate cyclase activity was linear with protein up to at least 4 mg. Inset: Van't Hoff plot (see text) of the dependence of K_{act} of (−)isoprenaline on temperature. K_{act} is the concentration of (−)isoprenaline which activates adenylate cyclase to 50% of maximum. Each point represents the mean of three experiments done in duplicate ± the s.e.m. The correlation coefficient (r^2) was 0.985, calculated by the method of least squares, and the enthalpy change of the reaction ($\Delta H°$) was −9.9 kcal mol^{-1}, calculated from the slope of the line ($-\Delta H°/R$; see text).

Table 2 Thermodynamic parameters of ligand binding to the β-adrenergic receptor of turkey erythrocytes

	$\Delta G°$ (kcal mol^{-1})	$\Delta H°$ (kcal mol^{-1})	$\Delta S°$ (entropy units)
Agonists			
(−)isoprenaline	−9.39	−13.39	−12.9
(−)noradrenaline	−7.91	−18.86	−35.3
(−)adrenaline	−7.50	−12.75	−16.9
Partial agonists			
Soterenol	−8.23	−7.84	+1.26
Fenoterol	−7.81	−6.06	+5.65
Salmefamol	−7.28	−8.86	−5.10
Metaproterenol	−6.78	−10.83	−13.06
Terbutaline	−6.19	−4.13	+6.65
Antagonists			
(−)propranolol	−12.51	−3.85	+27.9
MK-950	−12.32	+0.81	+42.3
IPS-339	−12.30	+0.25	+40.5
Pindolol	−11.85	−5.08	+21.8
Zinterol	−9.13	−3.06	+19.6
Metoprolol	−8.36	−0.66	+24.8
Sotalol	−8.21	−2.15	+19.5
H 35/25	−7.96	−1.83	+19.8
Butoxamine	−7.71	−1.08	+21.4
OPC 2009	−7.55	−0.90	+21.5
Atenolol	−7.49	−3.47	+13.0
Practolol	−7.46	+3.92	+36.7

Assays were carried out in 0.09 M NaCl containing Tris-Cl (0.012 M, $pH = 6.8$). The changes in free energy, ($\Delta G°$), enthalpy ($\Delta H°$) and entropy ($\Delta S°$) of binding at 37 °C were calculated as described in the text.

an induced conformational change of the receptor protein or immediately surrounding membrane components. The net decrease in entropy would be the sum of the increase in entropy associated with binding and the larger decrease resulting from conformational changes. The large net decrease in enthalpy may primarily reflect the lower enthalpy of the particular conformational state of the receptor induced by the agonists.

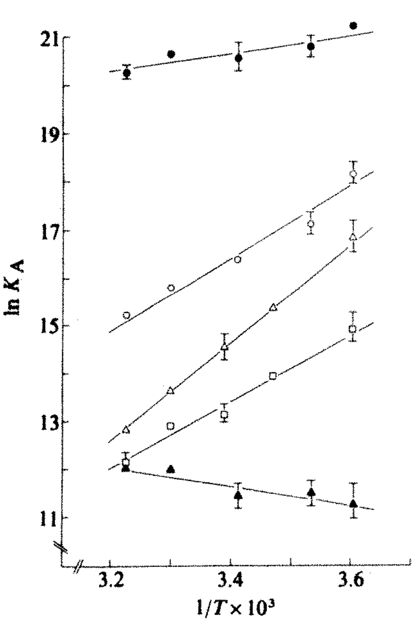

Fig. 3 Van't Hoff plots of the dependence of K_A on temperature. The data shown are for (−)propranolol (●), (−)isoprenaline (○), (−)noradrenaline (△), (−)adrenaline (□) and practolol (▲). K_A values were calculated from the equilibrium dissociation constants ($K_A = 1/K_D$) determined as described in Table 1. Each point represents the mean of 3–10 determinations done in duplicate ± the s.e.m. The s.e.m. of points with no error bars indicated were less than the size of the symbols. The dissociation constants for IHYP (in pM) were: 42 (37 °C), 33 (30 °C), 27 (24 °C), 23 (20 °C), 23 (15 °C), 22 (10 °C) and 20 (1 °C). The density of binding sites did not vary with temperature. The slope of the line is equal to $-\Delta H°/R$ (see text).

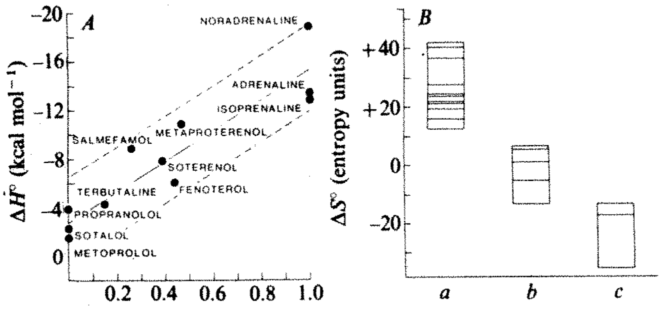

Fig. 4 Relationship between the enthalpy and entropy of binding and efficacy. *A*, The changes in enthalpy for the binding of ligands are plotted as a function of their efficacy. Efficacy was measured as the maximal activation of adenylate cyclase at 37 °C and is expressed as activation relative to the maximal activation by 30 μM (−)isoprenaline. Only three representative antagonists are shown. The solid line represents a linear regression analysis of the data shown. The correlation coefficient is 0.916. The dashed lines show the range of the values observed. *B*, The changes in entropy for the binding of the ligands in Table 2 are plotted as a function of antagonist (*a*), partial agonist (*b*) and full agonist (*c*) properties. Each horizontal line represents a ligand. The average entropy change for each group is: antagonists, +25.7 ± 2.7 EU; partial agonists, −0.92 ± 3.57 EU; full agonists, −21.7 ± 6.19 EU; The mean values of antagonists, partial agonists and full agonists differed from one another ($P < 0.01$). A plot of $\Delta S°$ against efficacy (not shown) gave a correlation coefficient of 0.888.

A two-step model similar to that proposed for agonist interactions with the nicotinic receptor[16,17] may be useful for β-adrenergic receptors.

$$L + R \overset{1}{\rightleftharpoons} LR \overset{2}{\rightleftharpoons} LR' \tag{4}$$

In this model, L is the ligand, R the receptor and R' the agonist-induced state of the receptor protein. Antagonists would participate only in reaction 1, with little or no change in enthalpy and large increases in entropy. Agonists and antagonists may bind in reaction 1 with similar themodynamic components; however, because of structural differences agonists are able to induce a conformational change of the receptor to R'. This conversion, reaction 2, would have negative enthalpy and entropy components. A large decrease in enthalpy could compensate for an unfavourable decrease in entropy and give reaction 2 a net negative free energy change, allowing it to occur. The extent of conversion to the R' state, the receptor conformation which leads to activation of adenylate cyclase, would be reflected in the efficacy of the particular ligand. This is consistent with the observed correlation between efficacy and both $\Delta H°$ and $\Delta S°$.

General use for the thermodynamic approach

Although the turkey erythrocyte membrane preparation is widely used for the study of the β-adrenergic receptor/adenylate cyclase system, the pharmacological specificity of β-adrenergic receptors on these cells is different from that of mammalian β_1- or β_2-adrenergic receptors[12]; there is no effect of GTP on agonist binding[18], and 'desensitisation' does not occur[19]. Despite these differences, the results and interpretation discussed above may be generally applicable. Preliminary results have shown differences in the thermodynamics of agonist and antagonist binding to β-adrenergic receptors of rat cerebral cortex and heart similar to those reported here (unpublished results). There is also physiological evidence that β-adrenergic receptors in several tissues have higher affinities for agonists at lower temperatures than at more physiological temperatures[7–10,20–22].

Various treatments cause agonist-specific changes in β-adrenergic receptor affinity. As affinity is a function of changes in the enthalpy and entropy of binding, alterations in affinity will reflect changes in these components. Thus, thermodynamic analyses of the agonist-specific changes in affinity which have been reported following cell disruption[23,24] and membrane solubilisation[6,25] and treatment with guanine nucleotides[26,27] or divalent cations[28] may contribute to our understanding of the molecular mechanisms involved in these changes.

Finally, temperature-dependent changes in apparent affinity have also been observed for the α-adrenergic receptor[29], histamine receptor[30,31] and for the affinity of ^{3}H-flunitrazepam for benzodiazepine receptors[32]. Thus, thermodynamic analysis may be a generally useful tool for examining the molecular events involved in hormone–receptor interactions.

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Chicken ovalbumin contains an internal signal sequence

Vishwanath R. Lingappa, Jaisri R. Lingappa & Günter Blobel
Laboratory of Cell Biology, The Rockefeller University, New York, New York 10021

Ovalbumin is shown to contain an internal rather than an amino-terminal signal sequence. This internal signal can be recovered in a tryptic fragment which comprises residues 229–276 of mature ovalbumin and contains a region of striking sequence homology to amino-terminal cleaved signals of two other oviduct secretory proteins. The isolated tryptic fragment is used as a probe to demonstrate that a signal receptor is present in membrane vesicles derived from the rough but not in those derived from the smooth endoplasmic reticulum.

THE information for translocation of polypeptides across membranes was proposed[1] to reside in a discrete portion of the polypeptide chain, termed the signal sequence. For many secretory proteins the signal has been shown to consist of a short-lived amino-terminal extension, 15–30 amino acid residues long[1], that is cleaved from the chain during its synthesis and translocation across the rough endoplasmic reticulum (RER) membrane. Although the primary structure of the signals for various secretory proteins differs, there is circumstantial evidence that they all share certain features in their secondary structure[1].

In addition to secretory proteins, certain integral membrane proteins have been shown to contain a signal sequence that is functionally equivalent to that of secretory proteins[2]. These data suggest the existence in the RER of only one polypeptide transport system (translocator) that mediates transfer across the membrane in response to a signal which is common to secretory and certain integral membrane proteins.

It has been anticipated that cleavage of the signal is not an obligatory step for translocation of proteins across the RER membrane[3]. Ovalbumin seemed to provide a first example as it was found to be synthesised without the typical transient signal sequence[4]. Moreover, data obtained from *in vitro* translocation and competition experiments[5] provided strong evidence for the existence in *de novo* synthesised ovalbumin of an uncleaved signal sequence that was functionally identical to the cleaved signals of other secretory proteins. Like its cleaved counterpart the uncleaved signal of ovalbumin is expressed only by nascent but not by the completed, presumably folded chain[5]. Consequently, it was reasoned[5] that mature and secreted ovalbumin should contain a 'latent' signal that might be uncovered after denaturation or limited splitting of the molecule. The data presented here demonstrate that this hypothesis was correct, and, surprisingly, that ovalbumin contains an internal rather than an amino-terminal signal sequence.

Detection of signal activity in mature ovalbumin

To determine signal activity we measured the extent to which ovalbumin or its fragments are able to inhibit competitively *in vitro* translocation of nascent bovine pre-prolactin into dog pancreas microsomal vesicles[5]. The assay system consists of a cell-free protein-synthesising system (in the present case, a nuclease-treated rabbit reticulocyte lysate[6]) programmed with bovine pituitary mRNA and supplemented with ribosome-stripped and nuclease-treated microsomal vesicles from dog pancreas[7]. In the absence of microsomal vesicles, translation of bovine pituitary mRNA yields a major product (Fig. 1*a*, lane 1), previously shown[8] to be pre-prolactin (bovine pre-prolactin is

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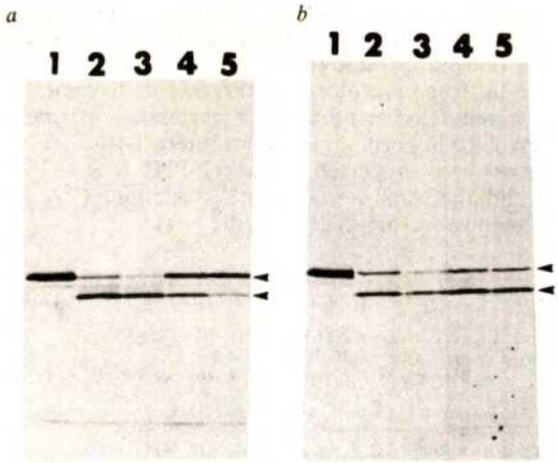

Fig. 1 Effect of unfractionated tryptic ovalbumin or serum albumin fragments on nascent pre-prolactin translocation. *a*, Bovine anterior pituitary mRNA (0.2 A_{260} units ml^{-1}) was translated in a nuclease-treated rabbit reticulocyte lysate containing 250 μCi ml^{-1} of ^{35}S-methionine (650 Ci $mmol^{-1}$) either in the absence (lane 1) or presence (lanes 2–5) of 1.0 A_{260} units ml^{-1} of nuclease-treated dog pancreas microsomal membranes[7]. Incubation mixtures also contained 8 mg ml^{-1} of native ovalbumin (lane 3, 8 mg ml^{-1}) of denatured ovalbumin (lane 4) or 0.5 mg ml^{-1} of tryptic ovalbumin fragments (lane 5). Preparation of denatured ovalbumin (OV_d) and tryptic ovalbumin fragments (OV_f) is described in Fig. 2 legend. Preparations of OV_d and OV_f were desalted[25] on Sephadex G-25 before being added to the incubation mixture. After incubation for 1 h at 25 °C, samples were prepared for polyacrylamide slab gel electrophoresis in SDS[8]. *a*, Shows an autoradiograph of the dried slab gel. Positions of pre-prolactin and prolactin are indicated by upper and lower arrowheads, respectively. *b*, As in *a*, except that equivalent concentrations of bovine serum albumin in native (lane 3), denatured (lane 4) or trypsin-digested (lane 5) forms was substituted for the corresponding ovalbumin forms.

prolactin plus an amino-terminal signal of 30 amino acid residues[8]). In the presence of microsomal membranes, however, prolactin is also synthesised (Fig. 1*a*, lane 2, and ref. 8), in amounts which depend on the concentration of microsomal vesicles: increasing concentrations of vesicles result in an increased synthesis of prolactin relative to pre-prolactin[8]. It has been demonstrated[8] that prolactin synthesis is the result of translation-coupled translocation of nascent pre-prolactin to the lumen of the microsomal vesicles where the signal is cleaved from the growing uncompleted chain. Following cleavage by signal peptidase on the luminal side of the microsomal vesicle, the nascent prolactin chain continues to grow and to be translocated until it is completed and entirely segregated within the lumen of the microsomal vesicle[8]. This *in vitro* synthesised and segregated prolactin is indistinguishable from mature prolactin[8]. Thus, because of their exclusive location—all pre-prolactin molecules outside the microsomal vesicles (sensitive to proteolysis) and all prolactin molecules inside the vesicles (resistant to proteolysis)—the ratio of prolactin to pre-prolactin is an accurate indicator for the extent of translocation[7,8].

As expected[5], mature native ovalbumin was ineffective as a competitive inhibitor for nascent pre-prolactin translocation, as the ratio of prolactin to pre-prolactin synthesised in the presence of mature native ovalbumin (Fig. 1*a*, lane 3) was comparable to that synthesised in its absence (Fig. 1*a*, lane 2). However, denatured ovalbumin was an inhibitor of nascent pre-prolactin translocation, significantly reducing the ratio of prolactin to pre-prolactin (Fig. 1*a*, lane 4). This inhibitory activity was dependent on the concentration of the added denatured ovalbumin and was lost on renaturation (data not shown).

Tryptic dissection of ovalbumin

The finding that mature ovalbumin molecules contain a latent signal activity that is uncovered on denaturation was of practical importance for our objective of undertaking a limited fragmentation of the ovalbumin molecule. Using this, we hoped to isolate an active fragment and localise the signal activity to a defined portion of the molecule. It would thus be possible to monitor rapidly the dissection process of the denatured ovalbumin molecule (by comparing its signal activity with that of the generated mixture of fragments) and thereby to establish conditions for its maximal fragmentation without a concomitant loss of signal activity. Such conditions were established by using trypsin as the dissecting tool. The tryptic ovalbumin fragments (see Fig. 2, lane 2) inhibited translocation of nascent pre-prolactin (Fig. 1*a*, lane 5).

Furthermore, neither mature (Fig. 1*b*, lane 3), denatured (lane 4) nor fragmented (lane 5) bovine serum albumin was able to inhibit translocations of nascent pre-prolactin. These results suggested that the inhibition of translocation observed with ovalbumin fragments was not a general property of secretory proteins or a nonspecific effect of hydrophobic peptides released from these globular proteins.

Purification and characterisation of an active ovalbumin fragment (OV_s)

The tryptic digestion mixture was subjected to conventional fractionation procedures described in detail in Fig. 2 legend.

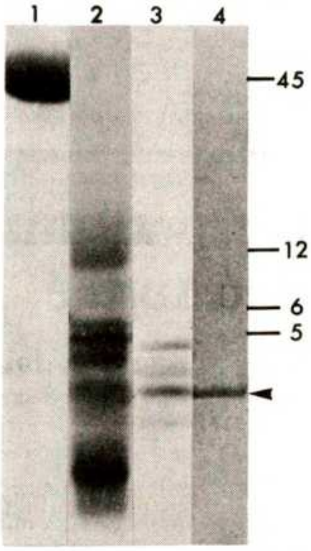

Fig. 2 Purification from trypsinised ovalbumin of a fragment with signal activity, monitored by polyacrylamide gel electrophoresis in SDS and 7 M urea. One gramme of chicken ovalbumin (Worthington) was dissolved in 20 ml of 0.25 M Tris-HCl, *p*H 7.5; 24 g of urea and 1.2 ml of 1.0 M dithiothreitol (DTT) were added and the solution volume was adjusted with water to 50 ml. After incubation at 37 °C for 1 h and at 100 °C for 15 min, the solution (containing 'denatured' ovalbumin (OV_d)) was diluted to 100 ml with water. 10 mg of trypsin–tosylphenylethylchloromethyl ketone (Worthington) were added every 6 h during 24-h incubation at 37 °C. Proteolysis was terminated by addition of phenylmethylsulphonyl fluoride (PMSF) to a final concentration of 2 mM. This solution of tryptic ovalbumin fragments was designated OV_f. Following isoelectric precipitation at *p*H 5.0 with glacial acetic acid, the precipitate was dissolved in 100 ml of 50 mM Tris-HCl, *p*H 7.5, 15 mM NaCl, 1 mM DTT and adjusted to *p*H 7.5 with 1.0 M NaOH. Ion exchange chromatography on DEAE-cellulose (25 ml bed volume) was carried out with 200 ml of a linear gradient of 15–750 mM NaCl containing 50 mM Tris-HCl, *p*H 7.5, and 1 mM DTT. Fractions (5 ml) were collected and assayed for signal activity, which was found to elute in a volume of ~40 ml with a peak at 400 mM NaCl. The material in this fraction was concentrated by another cycle of isoelectric precipitation at *p*H 5.0. The resulting pellet was dissolved in 5 ml of solution A (6 M urea, 50 mM Tris-HCl, *p*H 7.5, and 10 mM DTT) and applied to a column of Sephadex G-50 fine equilibrated with solution A. A major A_{280} peak eluted like a polypeptide of molecular weight (MW) ~8,000, in a total volume of 30 ml. This material is designated OV_{G50}. After dilution with water to 100 ml, an aliquot (1 ml) was subjected to polyacrylamide slab gel electrophoresis in 7 M urea (without SDS). Four distinct bands, similar in intensity and mobility to those shown in lane 3, were observed (data not shown). Individual bands were excised from the unstained gels and eluted by shaking, each into a volume of 5 ml of 5 mM Tris-HCl, *p*H 7.5. Following lyophilisation, each of the peptides was resuspended in 0.1 ml of 20 mM Tris-HCl, *p*H 7.5, and tested for signal activity. One of the peptides, designated as OV_s, was found to be active (see Fig. 3). Lane 1, 100 μg denatured ovalbumin (OV_d), note the absence of detectable degradation products; Lane 2, 20 μl of trypsin-digested ovalbumin (OV_f); Lane 3, 20 μl of OV_{G50}; Lane 4, 10 μl of OV_s. Numbers to the right of lane 4 refer to size ($\times 10^3$) of MW markers.

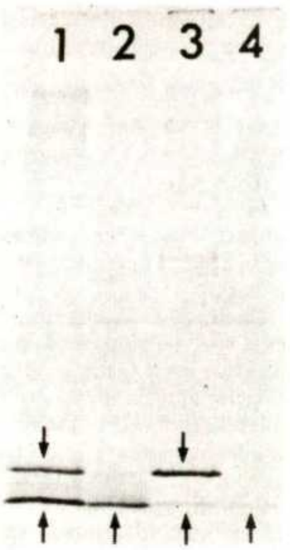

Fig. 3 Inhibition of nascent pre-prolactin translocation by a tryptic ovalbumin fragment (OV_s). Bovine pituitary mRNA was translated in the presence of microsomal membranes as described in Fig. 1 legend. OV_s (see lane 4 of Fig. 2) was either absent (lanes 1 and 2) or included (lanes 3 and 4) in the incubation mixture. Following termination of incubation for protein synthesis, aliquots (lanes 2 and 4) were subjected to post-translational proteolysis (incubation with 200 $\mu g\ ml^{-1}$ each of trypsin and chymotrypsin for 1 h at 0 °C in the presence of 10 mM $CaCl_2$). Proteolysis was terminated by the addition of Trasylol[2] and 1 mM PMSF followed by preparation for SDS-polyacrylamide slab gel electrophoresis. The figure shows an autoradiograph of the dried slab gel. Downward pointing arrow indicates pre-prolactin, upward pointing arrow indicates prolactin.

Table 1 Amino acid composition of OV_s compared with that of mature ovalbumin (OV_{1-385})[9] and selected tryptic ovalbumin fragments ($OV_{229-276}$ and OV_{1-46})

Amino acid	OV_s	$OV_{229-276}$	OV_{1-46}	OV_{1-385}
A	1.5	1	7	35
C	0.0	0	2	6
D/N	2.9	3	3	31
E/Q	9.4	9	3	48
F	2.0	2	4	20
G	2.1	2	3	19
H	0.0	0	2	7
I	2.0	3	4	25
K	0.7	1	3	20
L	7.7	8	3	32
M	3.2	3	3	16
P	2.2	2	1	14
R	0.8	1	0	15
S	5.5	6	3	38
T	2.6	3	0	15
V	3.2	3	3	31
W	(+)	1	0	3
Y	0.0	0	2	10

Purification of the signal activity was followed by assaying fractions for their inhibitory activity on nascent pre-prolactin translocation (data not shown) and by electrophoresis of active fractions in polyacrylamide gels containing urea and SDS (Fig. 2).

We succeeded in isolating a tryptic fragment which was pure by the criterion of its electrophoretic mobility as a single band (Fig. 2, lane 4) and which contained signal activity (Fig. 3); it was therefore designated OV_s. The presence of OV_s in the *in vitro* translocation system reduced the ratio of prolactin to pre-prolactin (compare lane 3 with lane 1 in Fig. 3). The increased amounts of pre-prolactin that were synthesised in the presence of OV_s were clearly extra-vesicular as they were digested by post-translational proteolysis (Fig. 3, lane 4). This result ruled out the possibility that OV_s acted as a competitive inhibitor not of nascent pre-prolactin translocation but of signal peptidase (in which case one would have detected pre-prolactin molecules within microsomal vesicles, protected from post-translational proteolysis).

OV_s was then subjected to amino acid analysis. When compared (Table 1) with the amino acid composition of ovalbumin, OV_s differed by an absence of Cys, Tyr and His residues, a relative abundance of Leu, Thr, Glu/Gln, Met, Ser and Ile residues, and a paucity of Ala residues. Furthermore, OV_s contained one Lys and one Arg residue, suggesting the presence of one trypsin site that had not been cleaved.

Based on its distinct amino acid composition, OV_s could be readily and unambiguously identified within the known sequence[9] of the ovalbumin molecule (Table 1). Thus, OV_s represents a limited tryptic fragment which contains 48 amino acid residues, beginning with Ile 229 and ending with Arg 276, and contains an uncleaved tryptic site represented by Lys 263. The relative resistance of this site to cleavage may be due to the presence of a glutamyl residue at position 262.

The localisation of signal activity in an internal rather than the amino-terminal region of mature ovalbumin was unexpected. The purification of OV_s from other tryptic fragments of ovalbumin was based on the premise that its ability to reduce the ratio of prolactin to pre-prolactin synthesised in an *in vitro* translocation system was due to competition (with the amino-terminal and cleavable signal of nascent pre-prolactin) for a site on the RER translocator; this premise needed to be substantiated to rule out an alternative mode or site of action of OV_s.

Homology of OV_s to signals of other chicken oviduct secretory proteins

Convincing evidence for the notion that OV_s contains the equivalent of the ovalbumin signal was obtained from a structural comparison (Fig. 4) of OV_s with the amino-terminal and cleaved signals of two other hen oviduct secretory proteins, ovomucoid and lysozyme[10,11]. There is clearly a significant degree of sequence homology. Particularly striking are two clusters of homology, each comprising three hydrophobic residues. There are also clusters of amino acid residues (indicated in bold face, Fig. 4) differing in their genetic code by a single base. Furthermore, the penultimate Gly residue of the two cleaved signals readily aligns with a Gly residue in OV_s. However, ovalbumin is not cleaved at this site. A possible explanation for this could be the absence of Cys or of Cys-Gly, located between the two hydrophobic homologous clusters in the case of the two cleaved signals but absent in OV_s (Fig. 4). It is likely[1,12] that this region constitutes the site of a β-turn or another secondary structural feature that is important for recognition by signal peptidase. Consistent with this reasoning is the recent demonstration[13] that a replacement of a glycyl residue by an aspartyl residue at position 14 of the 20-residue-long signal of lipoprotein in an *Escherichia coli* mutant results in the loss of the option to be cleaved but not of the ability to be translocated.

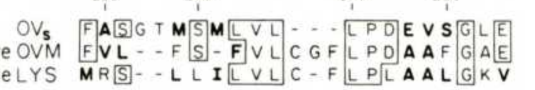
235
240
245
250
OVs F A S G T M S M L V L - - - L P D E V S G L E
pre OVM F V L - - F S - F V L C G F L P D A A F G A E
pre LYS M R S - - L L I L V L C - F L P L A A L G K V

Fig. 4 Homology of OV_s to amino terminal cleaved signals of other chicken oviduct secretory proteins. The location of OV_s within the ovalbumin molecule was deduced from its distinctive amino acid composition (see Table 1) and the known[9] sequence of ovalbumin. Signal sequences[10,11] of pre-ovomucoid (pre OVM) residues 7–24 and of pre-lysozyme (pre LYS) residues 1–18 were each aligned to maximise homology to ovalbumin residues 234–254 within OV_s. Homologous residues between OV_s and pre OVM or between OV_s and pre LYS are boxed. Residues involving a single base change are indicated in bold face. Cleavage site of pre OVM and pre LYS is after glycine and is aligned with Gly 251 of ovalbumin.

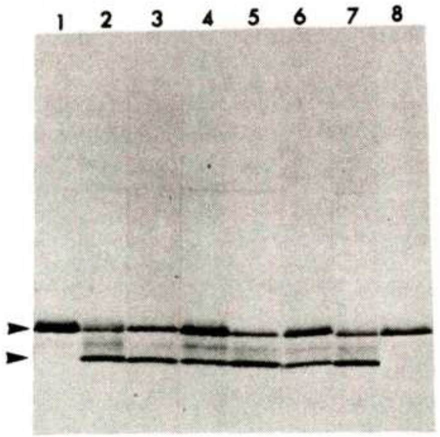

Fig. 5 RER-specific, competitive inhibition of nascent pre-prolactin translocation by $\mathrm{OV_s}$. Bovine pituitary RNA (0.2 A_{260} units ml^{-1}) was translated in the nuclease-treated reticulocyte lysate in the absence or presence of various membrane fractions and $\mathrm{OV_s}$. Membrane fractions were derived either from isolated rough or smooth microsomes (both from dog pancreas)[26] after removal of ribosomes and nuclease treatment[7] and are referred to in the text as RER or SER, respectively. Numbers refer to concentration in A_{260} units ml^{-1} (RER) or A_{280} units ml^{-1} (SER or $\mathrm{OV_s}$). Lane 1, neither membranes nor $\mathrm{OV_s}$ added. Lane 2, 1.0 RER, no $\mathrm{OV_s}$. Lane 3, 1.0 RER, 0.5 $\mathrm{OV_s}$. Lane 4, 1.0 RER, 1.0 $\mathrm{OV_s}$. Lane 5, 2.0 RER, 1.0 $\mathrm{OV_s}$. Lane 6, 1.0 RER, 1.0 SER, 1.0 $\mathrm{OV_s}$. Lane 7, 1.0 RER, 1.0 SER, no $\mathrm{OV_s}$. Lane 8, 1.0 SER, no RER, no $\mathrm{OV_s}$. Upper and lower arrowheads are as in Fig. 1.

Mode and site of action of $\mathrm{OV_s}$

The structural homology of $\mathrm{OV_s}$ with the signals of lysozyme and ovomucoid strongly suggested the existence of a common target represented by a signal 'receptor' domain in one of the hypothetical subunits that was proposed to participate in the assembly of the functional translocator[1,14].

The following experiments were carried out to establish $\mathrm{OV_s}$ as a valid probe for this receptor. The approach taken was to vary the concentration of some of the components of the *in vitro* translocation system while keeping that of others constant, and to measure the effect on nascent pre-prolactin translocation[5]. Some representative data of these experiments are shown in Fig. 5, and a systematic and quantitative analysis is summarised in Fig. 6.

First, we established that $\mathrm{OV_s}$ is indeed a competitive inhibitor. Increasing concentrations of $\mathrm{OV_s}$ (Fig. 5, lanes 3 and 4, Fig. 6*a*) resulted in an increasing inhibition of nascent pre-prolactin translocation, and increased concentrations of nascent pre-prolactin at a constant concentration of $\mathrm{OV_s}$ resulted in increased synthesis of prolactin (Fig. 6*c*). The competitive inhibition of $\mathrm{OV_s}$ could be offset (Fig. 5, lane 3 versus lane 5 and Fig. 6*b*) by increasing the concentration of RER (microsomal vesicles derived from the rough endoplasmic reticulum).

To demonstrate the existence of a specific target for $\mathrm{OV_s}$ we took advantage of our finding here that SER (smooth microsomal vesicles which probably include Golgi membranes) is unable to translocate nascent pre-prolactin (Fig. 5, lane 8) or to compete with RER for translocation (Fig. 6*a*); this strongly suggests that SER does not possess a signal receptor. SER was therefore chosen as a control to rule out the possibility that $\mathrm{OV_s}$ affected translocation not by its interaction with a specific signal receptor but rather, for example, by nonspecific interaction with membrane lipids. If this were the case one would expect that increasing concentrations of SER would titrate $\mathrm{OV_s}$, thereby offsetting the competitive inhibition of $\mathrm{OV_s}$ on nascent pre-prolactin translocation. The data shown in Fig. 6*b* demonstrate that this was not so.

Timing of ovalbumin translocation

One implication of our results is that translocation of ovalbumin across membranes would be initiated relatively late in the course of synthesis of the ovalbumin molecule. The precisely timed addition of membranes to a cell-free translation system synchronised with respect to chain elongation by the addition of an inhibitor of initiation[15,16], should provide information on the timing of ovalbumin translocation. In such a system an amino-terminal signal would be expected to require an early addition of microsomal membranes whereas an internal signal sequence would allow a greater length of chain to be synthesised before the growing chain would lose the capacity for functional interaction with microsomal membranes resulting in chain translocation. Our results (Fig. 7) comparing the timing of translocation of prolactin and ovalbumin are consistent with these expectations. The half-time of membrane addition (defined as the time at which translocation is reduced by 50%) was 2.0 min for prolactin and 5.5 min for ovalbumin. The half-time of chain completion (defined as the time at which 50% of the chains are completed in the synchronised system in the absence of membranes) was 5.0 min for prolactin and 8.5 min for ovalbumin (data not shown). Thus, assuming, a linear rate of synthesis, about 45 amino acid residues are incorporated per chain per min. As pre-prolactin and ovalbumin consist of 228 and 385 residues, respectively, it follows that the critical chain length after which translocation is abolished is about 90 residues for prolactin and about 250 residues for ovalbumin.

Comments

These results demonstrate that mature ovalbumin contains a latent signal sequence that can be uncovered after denaturation and can be isolated from the molecule as a tryptic fragment ($\mathrm{OV_s}$) comprising amino acid residues 229–276. A striking sequence homology to the amino-terminal cleaved signals of two other hen oviduct secretory proteins (lysozyme and ovomucoid) is evident in part of $\mathrm{OV_s}$ comprising residues 234–253. It is likely, therefore, that the signal activity resides in this portion of the ovalbumin molecule. Our demonstration here that an internal signal can compete with the amino-terminal cleaved signals strongly suggests that the two have a common target, presumably a receptor domain of a protein translocator.

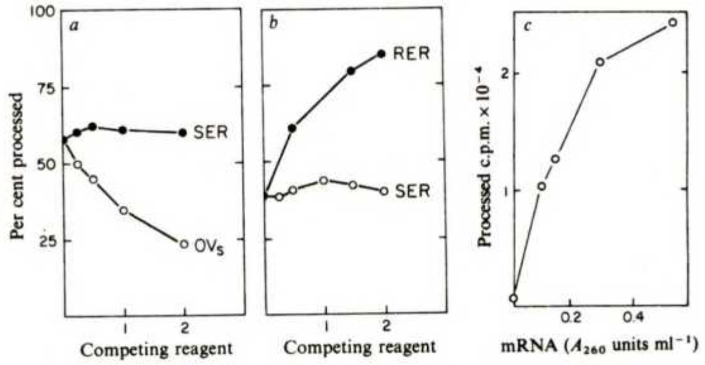

Fig. 6 Quantitative analysis of the effects of $\mathrm{OV_s}$ and SER on nascent pre-prolactin translocation. *a*, A constant amount of pituitary mRNA (0.2 A_{260} units ml^{-1}) was translated in the presence of a constant amount of RER (0.8 A_{260} units ml^{-1}) and increasing concentrations of either $\mathrm{OV_s}$ or SER. Unit concentration of competing reagent is 0.5 A_{280} units ml^{-1} for $\mathrm{OV_s}$ and 1.0 A_{280} units ml^{-1} for SER. Radioactivity in gel slices was determined as previously described[14]. The extent of translocation is expressed as percentage of radioactivity in the prolactin band over that in the pre-prolactin and prolactin bands. *b*, A constant amount of pituitary mRNA (0.2 A_{260} units ml^{-1}) was translated in the presence of a constant amount of $\mathrm{OV_s}$ (0.5 A_{280} ml^{-1}) and a minimum amount of RER (0.8 A_{260} units ml^{-1}). Increasing concentrations of either RER or SER were added as competing reagents in a unit concentration of 1.0 A_{280} units ml^{-1} for SER or 1.0 A_{260} units ml^{-1} for RER. *c*, Increasing concentrations of pituitary mRNA were translated in the presence of a constant amount of $\mathrm{OV_s}$ (1.0 A_{280} units ml^{-1}) and of RER (1.0 A_{260} ml^{-1}). The radioactivity in prolactin is shown as absolute c.p.m.

The existence of an internal signal sequence for the initiation of chain translocation has been previously postulated to explain the insertion into the RER of a viral integral membrane protein[17]. In this case, however, it was proposed[17] that the internal signal might be cleaved by signal peptidase, ultimately yielding two integral membrane proteins. The presence in ovalbumin of an uncleaved internal signal poses the intriguing problem of how nascent ovalbumin is actually threaded through the membrane. One possibility is translocation in the form of a loop, with the signal at its leading edge, in such a way that both the left (amino-terminal) and the right (carboxyl-terminal) wings of the molecule are translocated simultaneously, as the right wing of the molecule is being completed. Other conceivable mechanisms are cotranslational translocation primarily of the right wing followed by post-translational translocation of all or the remainder of the left wing of the molecule.

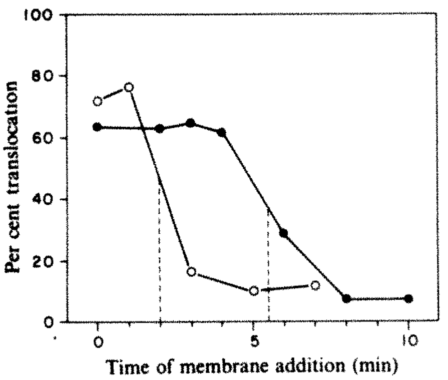

Fig. 7 Timing of translocation of prolactin and ovalbumin in a synchronised cell-free translation system. Protein synthesis was initiated in the reticulocyte lysate system. After 1 min of incubation, initiation of new chains was inihibited by the addition of 1.0 mM pactamycin[15]. At various times indicated on the ordinate, microsomal membranes (5.0 A_{260} units ml^{-1}) were added to aliquots of the translation mixture and protein synthesis allowed to proceed to completion. ^{35}S-Methionine-labelled translation products were analysed by SDS-polyacrylamide slab gel electrophoresis and radioactivity in individual bands was determined. Per cent translocation was expressed as either (○) per cent processed prolactin (PL/PL + prePL) or (●) per cent glycosylated ovalbumin ($\text{OV}_1/\text{OV}_1+\text{OV}_0$), as previously described[5].

Extrapolation of such considerations to the problem of how the polypeptide chains of integral membrane proteins (IMPs) achieve their often complex orientation with respect to the lipid bilayer offers interesting theoretical solutions. To define the relationship of the polypeptide chain with the lipid bilayer, IMPs can be classified[1] as monotopic (containing hydrophilic domain(s) exposed on only one side of the lipid bilayer) or bi- and polytopic (containing two or multiple hydrophilic domains exposed on both sides of the lipid bilayer). Two examples of bitopic IMPs have been shown[2,18] to be inserted into the membrane through an amino-terminal cleaved signal sequence that is functionally equivalent to that of nascent secretory proteins. To abrogate complete translocation of the nascent chain, the existence of a 'stop transfer' sequence has been postulated[2,19]. The combination of a cleaved amino-terminal signal and a stop transfer sequence further down the polypeptide chain would result in a bitopic orientation with the amino-terminal portion of the chain translocated and the carboxy-terminal portion untranslocated which, in fact, has been shown in these two cases[2,18]). Bi- or polytopic orientations could be achieved by an internal uncleaved signal sequence combined with a stop sequence. A stop on the right wing (see above) could result in a polytopic orientation whereby only an internal portion of the molecule is translocated, leaving substantial portions of both wings (containing amino and carboxy terminus) untranslocated. A stop on the left wing could result in a completely translocated right wing and an untranslocated left wing, leaving the amino-terminal portion of the molecule untranslocated. More complex polytopic orientations could be achieved by alternating multiple signal and stop sequences[17].

It is interesting that the last of the seven intervening sequences in the ovalbumin gene[20] is located between residues 254 and 255, that is, near the eliminated signal peptidase site. An intervening sequence near the signal peptidase site is also present in the gene for the light chain of IgG[21]. Thus, signal sequences and also stop transfer sequences could have evolved as a result of gene amplification and recombination[1].

The isolation of an internal signal is clearly of practical importance. OV_s has already been used here as a probe to demonstrate that its target is located in the rough and not the smooth endoplasmic reticulum. Experiments are in progress to study in detail the interaction of OV_s with the RER, in the hope that it can be used as an affinity ligand for the purification of its target.

Finally, the demonstration here of an internal uncleaved signal sequence with the same target in the RER as the amino-terminal cleaved signals, is relevant to currently used nomenclature. Retrospectively, it turns out that the term 'signal' was a more accurate choice[3] than designations such as leader sequence, pre-sequence or extra piece, all of which have structural implications such as amino-terminal location and/or cleavage. Thus, we propose that the term signal sequence be reserved to define a segment of the polypeptide chain, cleaved or uncleaved, which triggers protein translocation across a cellular membrane by virtue of its interaction with a specific translocator. Although it seems that a single translocator exists for the co-translational transfer of secretory and membrane proteins across the membrane of the RER, recent evidence indicates[22-24] that eukaryotic cells also contain several other specific translocators, localised to other distinct cellular membranes, for the recognition of structurally unique signals for the post-translational import of proteins from the cytosol into organelles.

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A structurally abnormal insulin causing human diabetes

H. Tager, B. Given, D. Baldwin, M. Mako, J. Markese & A. Rubenstein
Departments of Biochemistry and Medicine, The University of Chicago, Chicago, Illinois 60637

J. Olefsky, M. Kobayashi & O. Kolterman
Department of Medicine, The University of Colorado, Denver, Colorado 80262

R. Poucher
1201 West Laueta Street, Orange, California 92668

Insulin isolated from the pancreas of a diabetic patient with fasting hyperinsulinaemia showed decreased activity in binding to cell membrane insulin receptors and in stimulating cellular 2-deoxyglucose transport and glucose oxidation. Chemical studies suggest that the isolated hormone is a mixture of normal insulin and an abnormal variant which contains a leucine for phenylalanine substitution at position 24 or 25 of the insulin B-chain.

INSULIN RESISTANCE, a clinical syndrome identified by an individual's failure to respond normally to endogenously secreted and exogenously administered insulin, can lead to severe diabetes even when plasma levels of the hormone are elevated. In many patients, this 'resistance' is due to a target cell abnormality or to the presence of circulating insulin antagonists[1]. We and others have described conditions in which cellular defects involve decreased numbers of membrane-associated insulin receptors in peripheral tissues[2] or decreased cellular responsiveness at a step subsequent to hormone–receptor interaction[3]. Circulating antagonists which lead to insulin resistance include anti-insulin antibodies[4], anti-insulin-receptor antibodies[5,6] and counter-regulatory hormones at high levels[1,7]. Patients exhibiting elevated plasma ratios of proinsulin to insulin[8] may also seem to be insulin resistant, but in these individuals the peripheral concentration of the 5,800-molecular weight hormone is lower than indicated by direct radioimmunoassay, and the patients actually respond normally to their plasma concentrations of free insulin.

We recently identified a patient (R.C.) with diabetes and hyperinsulinaemia whose disease did not seem to fit any of the above descriptions. Our findings, which will be described in detail elsewhere, showed that (1) the patient exhibited a fasting plasma glucose level of >200 mg dl^{-1} (normal, 80–120 mg dl^{-1}) and a fasting, plasma insulin level of 70–120 μunits ml^{-1} (normal, 5–24 μunits ml^{-1}); (2) the ratio of proinsulin to insulin in the patient's plasma was normal; (3) the patient's plasma did not contain anti-insulin or anti-insulin-receptor antibodies, nor elevated concentrations of insulin antagonists such as growth hormone, glucagon or cortisol; (4) the patient responded to exogenously administered insulin in a normal manner; (5) monocytes isolated from the patient's blood contained normal numbers of insulin receptors; (6) insulin isolated from the patient's plasma by immunoaffinity chromatography had a decreased ability to bind to both cultured IM-9 lymphocytes and rat adipocytes; (7) the plasma insulin had lower than normal activity in stimulating glucose uptake and oxidation in rat adipocytes. Although the possibility of a detrimental modification of the patient's insulin by a plasma factor could not be excluded, we considered it most likely that the patient secreted an abnormal insulin, the structure of which had been modified by genetic mutation. During exploratory surgery for the examination of an abdominal mass with pancreatic involvement, a portion of the patient's pancreas was removed for histological study. The portion remaining after completion of the pathological examination was made available to us for biochemical analysis. This report describes the isolation and characterisation of the patient's pancreatic insulin.

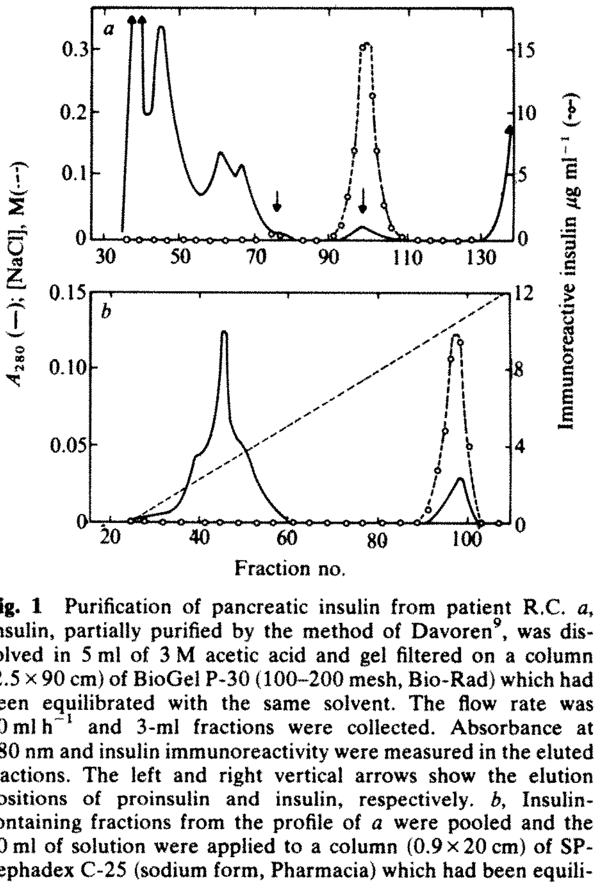

Fig. 1 Purification of pancreatic insulin from patient R.C. *a*, Insulin, partially purified by the method of Davoren[9], was dissolved in 5 ml of 3 M acetic acid and gel filtered on a column (2.5 × 90 cm) of BioGel P-30 (100–200 mesh, Bio-Rad) which had been equilibrated with the same solvent. The flow rate was 20 ml h^{-1} and 3-ml fractions were collected. Absorbance at 280 nm and insulin immunoreactivity were measured in the eluted fractions. The left and right vertical arrows show the elution positions of proinsulin and insulin, respectively. *b*, Insulin-containing fractions from the profile of *a* were pooled and the 30 ml of solution were applied to a column (0.9 × 20 cm) of SP-Sephadex C-25 (sodium form, Pharmacia) which had been equilibrated with 3 M acetic acid. The column was washed with 50 ml of the solvent and a linear gradient of NaCl dissolved in 3 M acetic acid (0–0.25 M, 200 ml of each) was applied to the column at a flow rate of 30 ml h^{-1}. Fractions containing 3 ml were collected and absorbance at 280 nm and insulin immunoreactivity were measured.

Isolation of insulin

A 3.1-g specimen of pancreas from patient R.C. was homogenised in acidified ethanol and the insulin partially purified by

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Table 1 Amino acid composition of isolated insulin and insulin fragments

	mol amino acid per mol peptide					
	Insulin		Desoctapeptide insulin		Heptapeptide B_{23-29}	
Amino acid	Found	Theory	Found	Theory	Found	Theory
Aspartic acid	3.2	3	3.2	3		
Threonine	2.8	3	1.2	1	1.0	1
Serine	2.5	3	2.9	3		
Glutamic acid	7.2	7	7.0	7		
Proline	1.1	1			1.0	1
Glycine	4.3	4	3.7	3	0.9	1
Alanine	1.0	1	1.3	1		
Cysteine	ND	6	ND	6		
Valine	3.9	4	3.6	4		
Isoleucine	1.9	2	1.6	2		
Leucine	6.8	6	6.0	6	0.6	
Tyrosine	4.0	4	3.0	3	1.0	1
Phenylalanine	2.3	3	0.9	1	1.4	2
Histidine	2.5	2	1.8	2		
Lysine	1.4	1			1.1	1
Arginine	1.1	1	1.0	1		

Peptides were hydrolysed under vacuum at 110 °C for 20 h in 6 M HCl containing a small amount of phenol. Analyses were carried out on a Durrum amino acid analyser. Relative amino acid concentrations were converted to nearest integer values to obtain the compositions reported in columns labelled 'Found'. Columns labelled 'Theory' show the known compositions of the normal human peptides. Cysteine content was not determined (ND).

the method of Davoren[9]. Material obtained by ether–alcohol precipitation was dissolved in 3M acetic acid and the resulting solution was gel-filtered on BioGel P-30 to yield the profile shown in Fig. 1*a*. Our finding that approximately 95% of the insulin-like immunoreactivity appeared as a single component having the size of native insulin (fractions 91–109) indicates a normal ratio of pancreatic proinsulin to insulin. To avoid additional methods of purification either requiring large amounts of material, such as crystallisation, or resulting in low yields, such as paper electrophoresis, we developed the specialised use of cation exchange chromatography for the final purification of the hormone. Fractions 94–105 from the profile of Fig. 1*a* were pooled and applied without concentration to a column of SP-Sephadex which had been equilibrated with 3M acetic acid. Application of a gradient of NaCl to the column resulted in the absorbance and immunoreactivity profiles shown in Fig. 1*b*. The peak of immunoreactive insulin was well separated from earlier eluting material which included the proinsulin C-peptide as well as other contaminating peptides. The yield of insulin, after pooling and desalting fractions 92–101, was approximately 50 nmol as measured either by absorbance at 275 nm or by colorimetric methods[10]. This value, which is equivalent to a yield of 98 mg per kg of tissue, represents the expected content of the hormone in pancreatic tissue[11].

To standardise the preparation further, identical amounts of the isolated insulin and monocomponent porcine insulin, as determined by the Lowry protein assay[10], were diluted in parallel and examined by radioimmunoassay[12]. As shown in Fig. 2, the immunometric potencies of the patient's insulin and porcine insulin were indistinguishable over a wide range of concentration. This result indicates the high degree of purity of the isolated insulin and validates the use of our radioimmunoassay in its measurement. Experiments not illustrated here also showed that the mobility of the patient insulin during polyacrylamide gel electrophoresis at *p*H 8.7 (ref. 13) or *p*H 4.5 (ref. 14) was indistinguishable from that of normal human insulin. As both gel systems are capable of resolving peptides having single charge differences, we could disregard the possibility that the preparation was contaminated by desamido- or arginyl-insulin, or that charged residues within the patient insulin had been modified. Glucagon was not detectable by sensitive radioimmunoassay in the final preparation.

Biological activity

The biological effectiveness of the pancreatic insulin from patient R.C. was compared with that of monocomponent porcine insulin on the basis of immunometric equivalency. This comparison is validated by the results of Fig. 2 and by our previous studies which have shown that porcine insulin and normal human insulin are equipotent when tested in any of the receptor-binding or biological assay systems used[3,25]. As illustrated in the experiments of Fig. 3, the patient insulin did not compete as well as normal insulin did for the binding of ^{125}I-labelled porcine insulin to cultured, human IM-9 lymphocytes or to isolated rat adipocytes. The shapes of the respective inhibition curves, however, were similar to those obtained using normal insulin. The decreased effectiveness of the patient insulin in these systems was confirmed on many occasions for the hormone isolated from both pancreas and plasma. Average potencies were 46 ± 8% of normal (average ± s.d.) for binding to IM-9 lymphocytes ($n = 9$) and 51 ± 8% of normal for binding to rat adipocytes ($n = 7$). Further experiments were designed to assess the biological activity of the insulin from patient R.C. Figure 4 shows that, whether activity was measured by stimulation of 2-deoxyglucose transport or by stimulation of ^{14}C-1-glucose oxidation, the patient insulin showed greatly decreased biological activity. Its potency, assessed by estimating that concentration causing half-maximal stimulation, was only 11 ± 5% ($n = 8$) and 12 ± 5% ($n = 14$) of normal in the two respective systems. Nevertheless, at high concentrations (10–50 ng ml^{-1}) the patient insulin was fully effective at stimulating these cellular processes. This decreased biological activity, as well as the decreased ability of the insulin to bind to membrane receptors, suggested that the pancreatic insulin from patient R.C. was structurally different from normal human insulin.

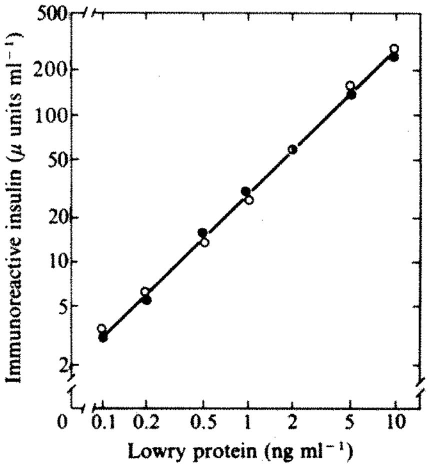

Fig. 2 Immunoreactive potency of the patient insulin. The protein concentration of the solution of patient insulin was determined by the method of Lowry[10] using a standard curve derived from porcine insulin. Amounts of the patient insulin and monocomponent porcine insulin corresponding to 1 μg of protein were diluted in parallel to give the concentrations shown on the abscissa. Aliquots (1 ml) of each solution were then subjected to the radioimmunoassay in standard conditions[12]. Averages of duplicate immunoreactivity determinations for the patient insulin (○) and monocomponent porcine insulin (●) are shown.

Chemical characterisation

Treatment of the purified patient insulin with 1-dimethylaminonaphthalene-5-sulphonyl chloride followed by subsequent analysis[19] led to identification of glycine and phenylalanine as the NH_2-terminal residues of the peptide. Amino acid analysis was carried out on the isolated peptide to probe a possible structural alteration in the patient insulin. As shown in the first column of Table 1, the compositional values obtained are very close to those expected for human insulin, with the exceptions that the content of leucine was high and that of phenylalanine low. The sources of the slightly elevated values for histidine and lysine are not known, but the presence of these residues in the patient insulin could be excluded because the peptide showed the expected electrophoretic mobilities during polyacrylamide gel electrophoresis. As NH_2-terminal analysis showed both phenylalanine and glycine, we could assign one residue of phenylalanine to position 1 of the insulin B-chain. In the simplest case, any decrease in phenylalanine content must then result from an amino acid substitution at position B_{24} or B_{25}. Fortuitously, these residues occur in the COOH-terminal octapeptide of the insulin B-chain, a region which is easily excised by proteolytic digestion.

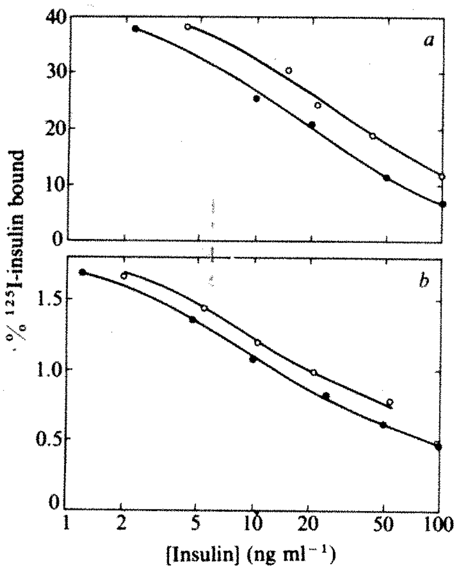

Fig. 3 Competition for the binding of ^{125}I-labelled porcine insulin to plasma membrane receptors by the patient insulin. *a*, Binding of insulin to cultured, human IM-9 lymphocytes; *b*, binding of insulin to isolated, rat adipocytes. Methods for the preparation of cells and for the binding studies using lymphocytes[16] and adipocytes[15,17,18] have been described. In each case, the potency of the patient insulin (○) was compared with that of monocomponent porcine insulin (●) at the concentrations shown.

The patient insulin (100 μg) was incubated at 30 °C for 3 h with 10 μg of tosylphenylalanine chloromethylketone-treated trypsin in 0.2 ml of 0.1 M *N*-ethylmorpholine brought to *p*H 8 with acetic acid. The resulting desoctapeptide insulin (lacking residues B_{23-30}), heptapeptide (containing residues B_{23-29}), and threonine (residue B_{30}) were separated by paper electrophoresis in the above *N*-ethylmorpholine buffer; peptides were subsequently eluted in 50% acetic acid. Amino acid analysis of the desoctapeptide insulin demonstrated, as expected, the presence of a single residue of phenylalanine (Table 1, column 3). The amino acid composition of the heptapeptide B_{23-29} (Table 1, column 5) shows values consistent with theory for glycine, tyrosine, threonine, proline and lysine. The peptide was deficient, however, by the equivalent of 0.6 residues of phenylalanine and contained the equivalent of 0.6 residues of leucine, an amino acid not expected from theory, nor found when we applied identical procedures to the preparation and analysis of the heptapeptide from porcine insulin. These results localise the leucine for phenylalanine substitution, which was suggested by the amino acid composition of the intact insulin, to the COOH-terminal octapeptide of the B-chain. Our finding of less than a full residue of leucine in the heptapeptide, however, suggests a limited degree of microheterogeneity in the isolated hormone. As we wished to conserve the remaining small amount of insulin for use as a structural probe of the insulin receptor, and as the amino acid substitution could only have occurred at one or the other of the two adjacent phenylalanine residues of the heptapeptide (B_{24} or B_{25}), we did not attempt to place the substitution in sequence.

Discussion

Our results show that the pancreatic insulin isolated from a patient who was resistant to endogenous insulin, but who responded normally to exogenous insulin, had a substantially lower than normal biological activity. These findings extend our observation that insulin isolated from the patient's plasma was of low potency and identify the defect in the hormone as occurring before its secretion. The leucine for phenylalanine substitution, which we localised in the COOH-terminal region of the B-chain of the patient insulin, represents a single base change which might have occurred by either of two point mutations, regardless of which of the phenylalanine codons were present in the gene sequence. This statistically favourable mutation undoubtedly results in the decreased biological effectiveness associated with the isolated insulin. Our finding of lower than integral values for the content of phenylalanine and leucine in the patient insulin, and a preliminary observation that the ^{125}I-labelled patient insulin is divisible into two components based on ability to bind to membrane receptors, suggest that the isolated hormone consists of normal human insulin and the mutant insulin in the approximate ratio 40:60. We consider that this mixture probably represents the products of the nearly co-dominant expression of two allelic genes in the heterozygous state; it is thus likely that identical maternal and paternal insulin genes are expressed simultaneously in normal individuals. In the absence of a more complete genetic analysis, however, we cannot exclude the possibility that the microheterogeneity, in

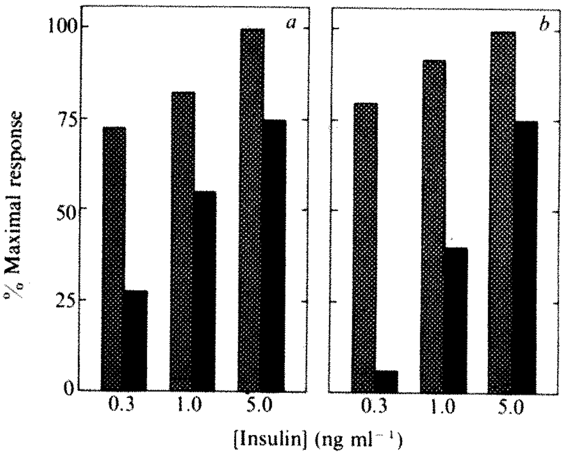

Fig. 4 Biological activity of the patient insulin. *a*, Stimulation of ^{14}C-2-deoxyglucose transport into isolated, rat adipocytes; *b*, stimulation of ^{14}C-glucose oxidation by isolated, rat adipocytes. Methods for the transport and glucose oxidation experiments have been described[12,15]. In each case, the potency of the patient insulin (solid bars) is compared with that of monocomponent porcine insulin (hatched bars) at the selected concentrations shown. Basal and maximally stimulated values for 2-deoxyglucose transport were 0.13 and 0.51 nmol per 2×10^5 cells per 3 min, respectively. Basal and maximally stimulated values for glucose oxidation were 3 and 61 nmol per 2×10^5 cells per h, respectively.

this selected case, results from the expression of two non-allelic genes, as is found for the two insulins of the rat[20,21].

Although we have not placed the proposed amino acid substitution in sequence, its localisation to position 24 or 25 of the insulin B-chain, as illustrated diagrammatically in Fig. 5, is particularly interesting. First, the B-chain sequence, Gly-Phe-Phe-Tyr, residues 23–26, has remained invariant during animal evolution, even when considering the hormones derived from the guinea pig[21] or the primitive hagfish[22]. No other sequence lacking cysteine residues and having comparable length seems to be as stable from an evolutionary point of view. Second, using various criteria, residues 23–26 of the COOH-terminal region of the B-chain have been placed in the active site of the hormone. Particularly important are the findings that the biological activity of modified insulins decreases progressively as from five to eight residues are removed from the COOH terminus of the B-chain[23] and that synthetic peptides containing residues B_{22-26} have low amounts of insulin-like activity[24]. The effects of replacing phenylalanine by leucine within this sequence seem to be quite specific, as this change has little effect on the general hydrophobic character of the region. Third, the COOH-terminal B-chain sequence containing residues 24–26 has been assigned to the negatively cooperative site for the interaction of the hormone with its receptor[25]. Fourth, this region of the B-chain has a crucial role in dimer formation within the zinc–insulin hexamer[26]. Note, however, that electron micrographs of islet tissue from patient R.C. revealed well defined crystalline inclusions typical of insulin-containing secretion granules. Other aspects of pancreatic and insular morphology were also unremarkable.

The low biological activity of the patient insulin (11–12% of normal), in comparison with its receptor binding activity (46–51% of normal), represents an unexpected result. Similar findings have been reported for the action and binding of hagfish insulin in mammalian systems (ref. 27 and S. Emdin, personal communication), but these findings have been questioned[28]. Notwithstanding this discrepancy, our results might be explained by a multi-step mechanism of insulin action allowing a degree of dissociation between receptor binding and hormone action, or by an antagonism of the action of the normal insulin by the abnormal variant in the preparation used. Because the apparent binding potency of the patient insulin corresponds well with that expected from the amount of normal insulin present in the mixture, the latter possibility seems the more likely. The very low biological activity of the patient insulin further suggests that this proposed antagonism occurs in a multiplicative way; that is, one molecule of the variant apparently inhibits the action of several molecules of normal insulin. This model would have important implications in the study of the mechanism of insulin action.

The relationship between the insulin variant and our patient's diabetes is important. Considerations of spare insulin receptors might have predicted a physiological compensation by the secretion of additional amounts of the hormone. The antagonistic behaviour of the variant in the secreted hormonal mixture suggests, however, that such compensation might not have been possible *in vivo*. It is likely that the increased ratio of active insulin to the variant produced by the insulin tolerance test was crucial in correcting the patient's glucose intolerance in our clinical studies. Our description of an abnormal insulin suggests the usefulness of the cataloguing of diabetes in hyperinsulinaemic states in which the disease can be ascribed to (1) a mutation in the structural gene for (prepro)insulin, (2) incomplete conversion of proinsulin to insulin, (3) circulating antagonists to insulin, (4) defects in the insulin receptor site, and (5) defects in target cell responsiveness at a site distal to hormone–receptor binding. Identification of the biochemical abnormality in any individual thus requires a comprehensive evaluation of these various possibilities. We are now examining plasma insulin from other patients whose insulin resistance mimics that of patient R.C. Use of these insulins as naturally occurring analogues is likely to provide further insight into the structural requirements for the binding of insulin to its receptor and for its activation of cellular processes.

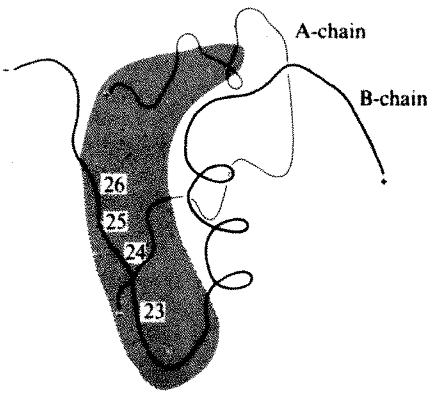

Fig. 5 Diagrammatic representation of the structure of insulin. The simplified drawing is derived from the crystallographic and other studies of Blundell *et al.*[29]. The biologically important, active site is indicated by hatching, and the positions of the invariant residues B_{23-26} (Gly-Phe-Phe-Tyr) are shown.

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letters

The high-latitude EUV source HD192273 as a low-mass binary

Harry L. Shipman & Gary A. Wegner

Physics Department, University of Delaware, Newark, Delaware 19711

The B star HD192273 was tentatively identified[1] as an extreme-ultraviolet (EUV) source. Photometry[2] and photographic spectra of this star show that the optical and EUV fluxes fit a model with $T_e = 27{,}000$ K and log $g = 4.8$, values reasonably close to those of a normal B2 V star. However, if HD 192273 is such a normal main-sequence star, its distance D is ~4 kpc, difficult to reconcile with the low line-of-sight column density ($<10^{18}$ cm^{-2} over the 4-kpc path), the low radial velocity (-24 km s^{-1}), and the high distance (~2 kpc) out of the galactic plane. Instead, we suggest that this object is an analogue of HZ22, an evolved, low-mass component of a binary[3], where the spectroscopic log g is consistent with a relatively small radius (0.1–1 $R_\odot$ and a small distance D of 100–200 pc. Such a suggestion can explain both the low H I column density and the small radial velocity.

Trimble's[4] calculations of the evolution of evolved binary components like HZ22 show that following mass exchange, stars with helium cores and thin hydrogen envelopes spend appreciable times (10^3–10^6 yr, with longer lifetimes at lower masses) near the He main sequence. Using Wegner's spectroscopic value of log $g = 4.8$,

$$\log R/R_\odot = -0.7 + 0.5 \log (M/0.1M_\odot) \tag{1}$$

Trimble's lowest-mass models with $M/M_\odot = 0.26$ fall reasonably close to the radius specified by equation (1). The spectroscopic temperature of 27,000 K specifies the monochromatic Eddington flux as 2.52×10^{-4} erg cm^{-2} Hz^{-1} (ref. 5). Adopting the relation between flux, magnitude, radius, and distance described in ref. 6, using equation (1) and taking $V = 8.827$, we obtain:

$$D = 80(M/0.1M_\odot)^{1/2} \text{ pc} \tag{2}$$

The precise value of D depends more on M and less on T and log g. We suggest that M is a few tenths of a solar mass giving $D = 100$–200 pc.

Wegner[2] derived a maximum H I column density of 5×10^{17} cm^{-2} from fitting a $T_e = 28{,}630$ K model to the observations of the EUV flux. Some exploration of the sensitivity of the upper limit to assumptions regarding T_e and log g is warranted, because the dependence of the EUV flux on T_e is quite steep, (the flux at 600 Å increases by a factor of 100 between $T_e = 27{,}500$ K and $T_e = 32{,}500$ K in Mihalas' models)[7]. A maximal temperature of 32,500 K, log $g = 4$, and a non-LTE model maximises the EUV flux at the stellar surface in a way consistent with the optical observations. Such a model gives $F(600\text{ Å})/F(5{,}500\text{ Å}) = 0.27$ at the star, which can be reduced to the observed value (at the Earth) of 0.0034 if the optical depth at 600Å to HD192273 is 2.

Such an optical depth corresponds to a column density of 10^{18} H I atoms cm^{-2} using the cross-sections of Cruddace *et al.*[8]. This limit is insensitive to the details of the analysis. No matter what kind of an EUV emitter this source is, the optical depth to it cannot be too high without making it invisible.

Is this interstellar column density reasonable, given current conceptions and observations of the interstellar medium? Consider two models: (1) HD192273 is a normal, main-sequence B star with $M_V = -4$ at a distance of 3.6 kpc (ref. 9) and (2) the present suggestion that it is a low-mass binary at a distance of 100–200 pc. Bohlin *et al.*[10] provides a survey of interstellar N(H I) based on observations of Lyα. Their Fig. 5 contains observations of stars far from the galactic plane, showing positive detections of N(H I) exceeding 10^{20} cm^{-2} for seven high-latitude stars with distances exceeding 100 pc. Were HD 192273 a normal B star, its distance of 3.6 kpc would place it far beyond all the stars observed by Bohlin *et al.* We therefore conclude that model (1) is inconsistent with the data from interstellar Lyα observations.

However, if HD192273 were much closer, the interstellar column density required is compatible with the observations of other EUV sources. The upper limit to the column density towards HD192273 is only slightly smaller than the values of the column density obtained for HZ43 [where $0.8 \times 10^{18} <$ N(H I) $< 2.5 \times 10^{18}$ (ref. 11)], and Feige 24 [where N(H I) can vary between 10^{18} and 5×10^{18} cm^{-2} (H.L.S., unpublished) or is less than 3×10^{18} (ref. 12).] The distances of HZ43 and Feige 24 are 60 pc (ref. 13) and 60–140 pc (ref. 14) respectively, comparable to the distance to HD192273 if it is a low-mass binary. Bohlin *et al.*[16] provide little information on such nearby objects, but the reasonably well established column densities towards HZ43, Feige 24, and the density towards HD192273 if model (2) is correct seem consistent with their observations, particularly considering that the neutral hydrogen is concentrated in a few clouds.

This interpretation of HD192273 is supported by two other pieces of information. Its spectroscopic log g, although uncertain, seems slightly high for a main-sequence B star. Furthermore, its low radial velocity would present problems were it 2 kpc from the galactic plane. Greenstein and Sargent[15] discuss a fairly large sample of similarly paradoxical high-latitude B stars. Their Table 4 provides an indication of the time required for stars with various radial velocities and at various heights z above the galactic plane to reach their present positions. FB180 and FB181, with z-distances of 1.6 and 1.9 kpc and $\rho'(z) = -31$ and -21 km s^{-1} respectively, are similar to HD192273 as a main-sequence B star with $z = 1.8$ kpc and $\rho' = -27$ km s^{-1}. (Here $\rho'(z)$ is the component of the radial velocity in the z-direction, corrected for solar motion.) Both FB180 and FB181 would take 4×10^7 yr to reach their present positions, and so we infer that a similar time scale applies to HD192273 if model (1) is correct. But as a main-sequence star, HD192273 has a lifetime of only 6×10^6 yr, and thus there is no obvious way that it could come to be where it is. However, if HD192273 is a low-mass binary component 100–200 pc away (model 2), the low radial velocity presents no problems.

This suggestion that HD192273 is a low-mass binary, combined with the spectroscopically confirmed binary nature of

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HZ22, prompts a speculation that many of the high-latitude O and B stars with low radial velocities and apparently large distances could be similar low-mass binaries at considerably smaller distances. This picture of HD192273 as a low-mass binary could be confirmed by a radial velocity study, although the absence of radial velocity variations would not rule out the present interpretation.

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A limit to the X-ray luminosity of nearby normal galaxies

D. M. Worrall, F. E. Marshall & E. A. Boldt

Laboratory for High Energy Astrophysics, NASA/Goddard Space Flight Center, Greenbelt, Maryland 20771

The origin of the extragalactic diffuse X-ray background is not well understood. It is isotropic to the extent that a galactic origin can be excluded. Indeed, even a halo of radius as large as 12 kpc could provide no more than 3% of the flux[1]. The spectrum between 3 and 50 keV fits a 40-keV thermal bremsstrahlung form which is suggestive of emission from a hot intergalactic medium[2]. This or some other truly diffuse mechanism may be responsible. If, however, discrete sources play a major part, we require either strong evolution of an already recognised X-ray emitting class, such as quasars, or as we consider here, a hitherto elusive population of low luminosity emitter.

Without invoking evolution, the largest background contributions are thought to derive from Seyfert galaxies and clusters of galaxies. Based on estimated local densities, the total background cannot be accounted for[2,3]. Consistency with fluctuation measurements of the diffuse background is only achieved if members of the additional non-evolved class of source, which would be required to complete the background, are sufficiently numerous and hence of low luminosity. Compatibility requires a volume density $\gtrsim 8\times10^{-3}h^3$ Mpc^{-3} (ref. 3), where h is the present Hubble constant in units of 100 km s^{-1} Mpc^{-1}. The diffuse background between 2 and 10 keV has an emissivity $\sim4.7\times10^{39}h$ erg s^{-1} Mpc^{-3}, which places an upper limit of $\sim6\times10^{41}/h^2$ erg s^{-1} on the source luminosity. (We quote luminosities between 2 and 10 keV to ease comparison with other work. However, the source and diffuse background data used here are both from the HEAO A-2 experiment and comparison will be made over the entire energy range for which we have a response, that is 2–60 keV; with highest efficiency 3–20 keV.)

Such weak extragalactic sources do not present a high detection probability for non-imaging proportional counter experiments. The requirements for source detection with the A-2 detectors on the HEAO 1 spacecraft are discussed in ref. 4. There is dependence on ecliptic latitude and consequent exposure but a 5σ detection threshold is $\sim2\times10^{-11}$ erg cm^{-2} s^{-1} (2–10 keV) requiring, for example, a 10^{40} erg s^{-1} emitter to be within ~2 Mpc. To date the only catalogued galaxies $<10^{42}$ erg s^{-1} are SMC, LMC, M31, Cen A and M82. Of these, the latter two are radio galaxies but are, probably, atypical of their class in general. The others are classed as normal galaxies and their X-ray luminosity is compatible with that of our Galaxy, estimated, from the summed emissivity of known sources, to be $\sim2\times10^{39}$ erg s^{-1}. Taking M31 and the Galaxy as standards, normal galaxies are estimated to contribute only a few per cent of the diffuse background[3,5,6]. However, as the most numerous extragalactic object they merit further examination in the search for a class of low luminosity X-ray source.

We report here results of a test of the hypothesis that normal galaxies are on average more luminous than our Galaxy and M31 and therefore form a candidate class of numerous low luminosity emitters which may fulfill requirements for contributing substantially to the diffuse background. The imaging experiments on the Einstein Observatory will measure at energies below ~4 keV where low-energy absorption and soft sources will limit comparison with the 2–60 keV background. As noted above, the comparison between source and background fluxes are made over the HEAO A-2 experiment energy range. But, where 2–10-keV source luminosities are given, a constant multiplicative factor suitable for a 9-keV thermal bremsstrahlung spectrum has been used.

Data from the A-2 detectors on the HEAO 1 spacecraft[7] were investigated for emission from positions coincident with normal galaxies selected from the *Second Reference Catalogue of Bright Galaxies*[8]. Selection required a velocity <1,100 km s^{-1} (that is $D<20$ Mpc for $H_0=55$ km s^{-1} Mpc^{-1}). Positions contaminated by catalogued X-ray sources were excluded. Since only data from the 1.5° × 3° field of view detectors were used, a source free region 3° each side of the galaxy along the scan direction and 5° in the perpendicular direction was demanded. Where galaxies were clustered the closest member was selected. Seventy-six galaxies satisfied requirements. A 25° field of scan angle about the galaxy position, accumulated over ~4 days about the one of maximum exposure, was fitted with a fixed source at the galaxy position and as many other sources as necessary to fit the remainder of the field. Negative values for the source count rate were allowed. After correcting for effective exposure for each fixed source a count rate distribution was found. This was consistent, to within 1σ errors, with zero. This result implies that

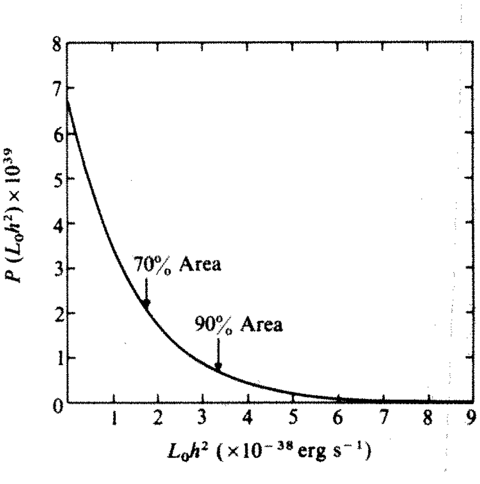

Fig. 1 The L_0h^2 probability function resulting from the application of a luminosity function $F(L, L_0)=(1/L_0h^2)\exp(-L/L_0h^2)$ to the sample of 76 normal galaxies. The result fits the indicated exponential function. The values of L_0h^2 bounding 70% and 90% of the area are shown, that is the upper limits to L_0h^2 at these respective confidence levels. $P(L_0h^2)\propto\exp(-L_0h^2/1.46\times10^{38})$.

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we can only determine upper limits to the average luminosity for various model luminosity distributions.

The simplest model which can be examined is one in which all galaxies are assumed to have a standard luminosity, Lh^2. Then the probability of observing the ith member of the 76 galaxies with its measured luminosity, L_ih^2, and associated standard deviation, σ_i, is $P_{si}(Lh^2) = (\sigma_i(2\pi)^{1/2})^{-1} \exp(-z_i^2/2)$, where $z_i = (L_i - L)h^2/\sigma_i$. Hence the likelihood function for the measured luminosities of the complete sample is $P_s(Lh^2) \propto \exp(-(\sum_i(z_i^2/2)))$. The values for σ_i were calculated from the standard deviation of the count rate distribution for the selected galaxies after this value had been confirmed to be consistent with values derived using random sample positions on the sky. The function $P_s(Lh^2)$ is of a gaussian form. The values of Lh^2 bounding 70% and 90% of the area are 1.5×10^{38} erg s^{-1} and 2.7×10^{38} erg s^{-1} respectively, which are the upper limits to the standard normal galaxy luminosity at these respective confidence levels. Even for a value of H_0 of 50 km s^{-1} Mpc^{-1} the results show that the average X-ray luminosity of normal galaxies is $\lesssim 10^{39}$ erg s^{-1}. This is a little lower than the X-ray luminosity of our Galaxy. However, we note that the average optical luminosity of the chosen galaxies in the B system is $(2 \times 10^9/h^2)L_\odot$ and the appropriate value for our Galaxy with which this should be compared is $11 \times 10^9 L_\odot$ (the mean orientation value estimated from the galactic pole value given in ref. 9).

More realistically, the luminosity function of the galaxies can be assumed to be an exponential, $F(L, L_0) = (L_0h^2)^{-1} \exp(-L/L_0h^2)$. The probability of observing the ith member of the 76 galaxies with its measured luminosity is now $P_i(L_0h^2) = \int F(L, L_0)P_{si}(Lh^2)\,\mathrm{d}L$. The product over all values of i gives the likelihood function for the complete sample. As shown in Fig. 1, it is approximately exponential. The 70% and 90% upper limits to L_0h^2 are now 1.8×10^{38} erg s^{-1} and 3.4×10^{38} erg s^{-1}, in reasonable agreement with the simple model.

The method generates a strong distance-dependent weighting factor. Because the answer is dominated by the closest 10 galaxies, selection effects may be important. To investigate this we divide the galaxies by distance into three bands. The number of galaxies per band increases with distance so that the three calculated luminosity upper limits are roughly equal. We find that they are ~3 times those quoted above. Thus we conclude that, to within a factor of 3, the 76 galaxies form a homogeneous class of which the closest 10 are not particularly anomalous. This is further confirmed in that the mean count rate for these 10 is within 1σ of the mean of the total sample.

Based on these results, we can make an h-independent comparison of the emissivity of galaxies with that of the diffuse background. The density of normal galaxies is calculated to be $\simeq 0.13h^3$ Mpc^{-3}. This uses a likely maximum value for the optical luminosity density of galaxies of $2.6 \times 10^8 hL_\odot$ Mpc^{-3} (see ref. 10) and the average luminosity for our sample as given above. For the detector fields of view we are using (defined as R15 in ref. 4) and applying the 90% upper limit criterion for the exponential luminosity distribution model, we find an emissivity of $< 2.7 \times 10^{45}h$ ct s^{-1} Mpc^{-3}. This is to be compared with the diffuse background emissivity of $\sim 3 \times 10^{47}h$ ct s^{-1} Mpc^{-3}. Thus normal galaxies contribute $\lesssim 1\%$.

Van Paradijs[6] has calculated the normal galaxy contribution to the X-ray background by modelling the X-ray luminosities of galaxy constituents. He estimates 0.5–2.8% (2–10 keV) for no evolution, but investigates several ways in which this value may be increased. While we cannot address those involving evolution, our calculations indicate that further non-evolutionary effects are not important.

By using a sample of objects we have investigated emission at luminosities lower than those for which we can study individual discrete sources and have shown that normal galaxies do not apparently provide the numerous low luminosity X-ray sources which could make up the 2–60-keV diffuse background. Indeed, upper limits suggest luminosities comparable with, or a little less than, that of the Galaxy. This is consistent with the fact that the average optical luminosity of the sample galaxies within ~20 Mpc is slightly lower than that of the Galaxy. An upper limit of ~1% of the diffuse background from such sources is derived.

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Radiolytic reduction of ceric ions in water DMSO mixtures and radioprotection

A. M. Koulkes-Pujo, M. Moreau & J. Sutton

CNRS and DRA/SRIRMa—CEN. Saclay B.P. No 2 91190 Gif Sur Yvette, France

It is convenient to classify mechanisms of radioprotection into those that involve fast radiation chemical processes and those in which the protection is the result of changes in the cell biochemistry. In the former, the added protectors have two principal effects: OH scavenging which reduces the radiosensitised damage and fast chemical repair processes involving hydrogen donation from the protector to the target radical. Typical of the first type of protector is dimethyl sulphoxide(DMSO)[1] because it is known to be a good OH scavenger ($k_{\mathrm{OH+DMSO}} = 5 \times 10^9$ mol^{-1} s^{-1}. (ref. 2). We have attempted to establish the redox properties of the OH radical adduct[3] as well as those of H_2O_2 or H and HO_2 radicals in water–DMSO mixtures. To this end we added to the solutions containing 0.5 M H_2SO_4, ceric ions at concentrations varying from 2.5×10^{-3} to 5×10^{-2} M. The Ce^{4+} reduction yield was determined as a function of the DMSO content in the solution, by irradiating the mixture with γ rays, in the presence or absence of air. The details of the experimental procedure will be given elsewhere. We report here that the yields (G) are independent of the Ce^{4+} concentration in the range studied and of the presence or absence of air, and depend only on the DMSO concentration.

Figure 1 shows the rate of disappearance of Ce^{4+} plotted against the DMSO concentration. One sees that the $G(-Ce^{4+})$ reaches a plateau value of 17.9 ± 0.2 between 0.3 M and 1.4 M in DMSO, or 2–10% by volume. This reduction yield is very high and has never been observed previously for any solute in aqueous systems. The yield of Ce^{4+} reduction in aqueous solution is 2.5 ± 0.3 and the reduction is accompanied by O_2 formation with a yield of 0.8. In the case of deaerated water–DMSO mixtures, there is no formation of O_2.

In acidified water, the intermediate species are H, OH and H_2O_2, with respective yields: $G_{\mathrm{H}} = 3.65$, $G_{\mathrm{OH}} = 2.95$, $G_{\mathrm{H_2O_2}} = 0.8$ (ref. 5). The reduction yield of Ce^{4+} is expressed as: $G(-Ce^{4+}) = G_{\mathrm{H}} + 2G_{\mathrm{H_2O_2}} - G_{\mathrm{OH}} = 2.3$ (ref. 4). If DMSO transforms both the escaping OH radicals as well as the hydrogen peroxide precursors into reducing radicals, so that each OH

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gives rise to three equivalents of reduction then the yield of reduction of Ce^{4+} may reach a value corresponding to the equation: $G(-Ce^{4+}) = G_H + 6\ G_{H_2O_2} + 3\ G_{OH} = 17.3$. The Ce^{4+} reduction reactions by these DMSO radicals would be stoichiometrically equivalent to following thermodynamically possible reactions of H_2O_2 and OH:

$$Ce^{4+} + H_2O_2 \rightarrow Ce^{3+} + HO_2 + H^+$$

$$Ce^{4+} + HO_2 \rightarrow Ce^{3+} + H^+ + O_2$$

$$Ce^{4+} + OH + H_2O \rightarrow Ce^{3+} + H^+ + H_2O_2$$

In these equations the yield corresponding to the capture of the radicals involved in the back reaction to form water is not taken into account. If DMSO scavenges these species the reduction yield should increase. Actually, the experimental value at the plateau is a little higher (17.9 ± 0.2) which may be the result of two simultaneous effects: an increase of the capture of the radicals coming from water which tends to increase $G(-Ce^{4+})$ and a greater participation of transitory species coming from DMSO radiolysis of which the overall yield may be smaller than that of water. This latter conjecture follows from the decrease of $G(-Ce^{4+})$ observed at DMSO concentrations from 1.4 M up to pure DMSO where the reduction yield becomes 13.9 ± 0.5. This value is still quite large suggesting that all the intermediates coming from DMSO also reduce Ce^{4+}. This yield then corresponds to $3\ G_{ox} + G_H$, G_{ox} being the yield of oxidising species coming from $DMSO^+$ created in the primary ionisation process. G_{ox} and G_H have been determined as equal to 3.6 and 2.1 respecitvely.[6]

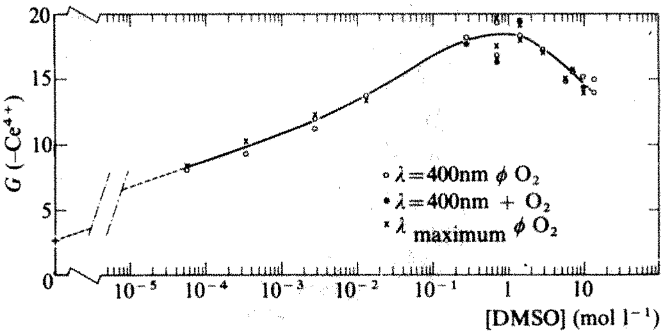

Fig. 1 The rate of disappearance of Ce^{4+} with the DMSO concentration.

It seems clear that the radicals formed by the reaction of DMSO with OH, H_2O_2, H or HO_2 all reduce Ce^{4+}. So DMSO not only acts as a good OH scavenger, suppressing an oxidising species, but the radical formed may also have reducing properties. This latter point could be important for the use of DMSO as a radioprotector. Another important point is the experimental observation of the absence of oxygen during this reduction reaction either by H_2O_2 or by HO_2. HO_2 like OH may give an adduct with DMSO which has reducing properties, but the reaction does not form O_2. This may also increase the radioprotective properties of DMSO by suppressing a very harmful radical HO_2 and by avoiding the formation of O_2 which is itself known to be a good radiosensitiser.

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The synthesis of fluor-analogues of natural asbestos by unidirectional crystallisation

M. Campbell, A. Maries & P. S. Rogers

Nuffield Research Group, Department of Metallurgy and Materials Science, Imperial College, London SW7, UK

A previous letter[1] reported a novel technique for the preparation of aligned fibrous crystals by drawing filaments of glass through a supported molten zone. The method was subsequently modified so that the filament is drawn from a melt through an orifice in the base of a conical platinum crucible[2]. This technique has now been successfully applied to the preparation of filaments consisting of aligned fibres of fluor-amphiboles, synthetic analogues of natural amphiboles with isomorphous replacement of hydroxyl by fluorine. These aligned fibrous crystals can be compared with natural amphibole asbestos.

Considerable work has been carried out, particularly in the USSR, to synthesise fibrous asbestos[3–5]. The fibres produced, 80–90% fluor-amphibole, occur as a mass of matted fibres with a short brush-like growth on the surface. Fibres from the brush-like growth can be up to 3.5 cm long, although those in the bulk are very much shorter; diameters are between 0.5 and 10 μm. The dynamic nature of the present technique enables filaments of axially aligned fibres to be drawn to whatever length is desirable, limited only by the dimensions of the experimental arrangement. Moreover a greater degree of control over the crystal diameter and phase assemblage is possible by varying such parameters as melt temperature, thermal gradient, crystallisation rate (via drawing speed), nozzle diameter and hence filament diameter. Figure 1 summarises the effect of certain parameters for a single crystallising phase, which have been discussed previously[2]. Briefly it can be seen that the temperature of crystallisation T_c, decreases with increasing drawing speed V

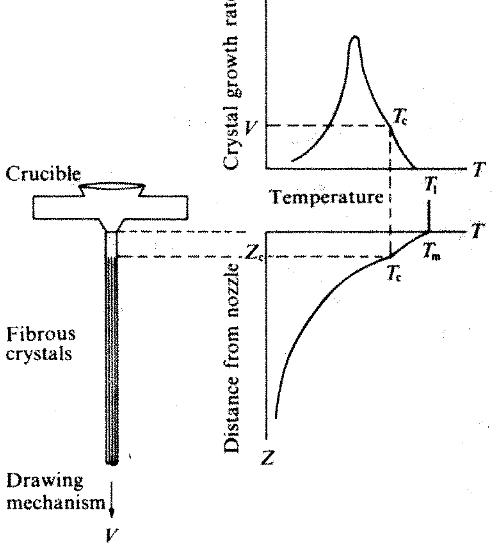

Fig. 1 Schematic diagram of apparatus for crystallisation from the melt (not to scale). Crucible lid with funnel has been omitted for clarity. T_m, T_l, T_c are melt, liquidus and crystallisation temperatures respectively; z_c, distance of crystal growth front from nozzle; V, drawing speed. Nozzle diameter is 0.9 mm and z_c is up to 1 mm.

Table 1 Batch compositions used successfully to produce fluor-amphiboles

Fluor-amphibole	Batch composition used	Liquidus	Crystalline phases in drawn filament
Fluor-magnesiorichterite $Na_2MgMg_5Si_8O_{22}F_2$	$Na_2Mg_6Si_8O_{20}F_6$	1,280 °C	(1) Water-swelling layered compound, probably fluor-tetrasilicic mica ($Na_2Mg_5Si_8O_{20}F_4$)[9] (2) Clinoenstatite (3) Small amount of fluor-magnesiorichterite
Fluor-tremolite $Ca_2Mg_5Si_8O_{22}F_2$	$Ca_2Mg_5Si_8O_{22}F_2.SiF_4$	~1,500 °C	(1) Clinoenstatite and diopside (as two solid solutions) (2) Fluor-tremolite
Fluor-richterite $Na_2CaMg_5Si_8O_{22}F_2$	$Na_2CaMg_5Si_8O_{22}F_2.Na_2SiF_6$	1,210 °C	(1) Fluor-richterite (2) Diopside and clinoenstatite additionally in certain conditions

until the maximum crystal growth rate is reached. The temperature of the melt T_m, which is above the liquidus T_l, determines the distance of the growth front from the nozzle (z_c).

The fluor-amphiboles that we have synthesised by this method are fluor-magnesiorichterite, fluor-tremolite and fluor-richterite; chosen because X-ray and optical data are readily available[6,7]. The first two have been produced here only in conjunction with pyroxene phases but the latter has been obtained as the only crystalline phase in the drawn filaments.

One of the main difficulties which arises when this method is applied to the synthesis of fluor-amphiboles is the loss of volatile fluorides from the melt; these are mainly SiF_4, with some NaF if Na is present and possibly some HF by hydrolysis. To reduce loss by volatilisation, the original cell[2] was slightly modified by welding a platinum lid on to the conical crucible, with a narrow funnel to allow recharging, closed at the top by a removable alumina stopper. Although this does not give a gas-tight seal, it does reduce the fluorine loss considerably. Starting materials were the anhydrous compounds MgO, SiO_2, Na_2CO_3, $CaCO_3$ and MgF_2, all of analytical purity. Batches were made up from these in proportions corresponding to the fluor-amphibole formula with a little extra fluorine and/or sodium. The most successful of the batch compositions used for each of the fluor-amphiboles is shown in Table 1, together with the crystalline phases produced and identified by powder X-ray diffraction.

In all cases where batch composition was close to the formula of fluor-magnesiorichterite the dominating phase was a water-swelling layered compound which contained twice the number of fluorine ions per silicate group as did the amphibole, at the expense of the latter. The amount of amphibole formed falls rapidly to zero as volatilisation reduces the F and Na available. A considerable amphibole yield was obtained from melts of composition close to that of fluor-tremolite, but always accompanied by pyroxene.

Reincorporation of sodium, together with calcium, into the batch reduced the liquidus temperature so that lower melt temperatures could be used and hence loss by volatilisation reduced, giving a very much higher amphibole/pyroxene ratio in drawn filaments. In fact over most of the temperature range possible for drawing, filaments were free from pyroxene, fluor-richterite being the only crystalline phase detectable by powder X-ray diffraction. The range of melt temperatures found possible for drawing was approximately 1,200 to 1,380 °C, whilst pyroxene appeared at the start of drawing above about 1,320 °C. However, the maximum drawing speed possible for crystallisation of fluor-richterite from this melt composition is somewhat low, 15 $\mu m\ s^{-1}$, compared with rates in excess of 60 $\mu m\ s^{-1}$ that have been used to draw filaments of the other two fluor-amphiboles. The X-ray pattern for fluor-richterite contained a few lines not recorded by Kohn and Comefero[7]. These, however, correspond to reflections permissible by the symmetry of the crystal structure (space group C2/m), and are in fact recorded for some other fluor-amphiboles and/or hydroxy-amphiboles[6-8]; moreover the reflections, including the 'extra'

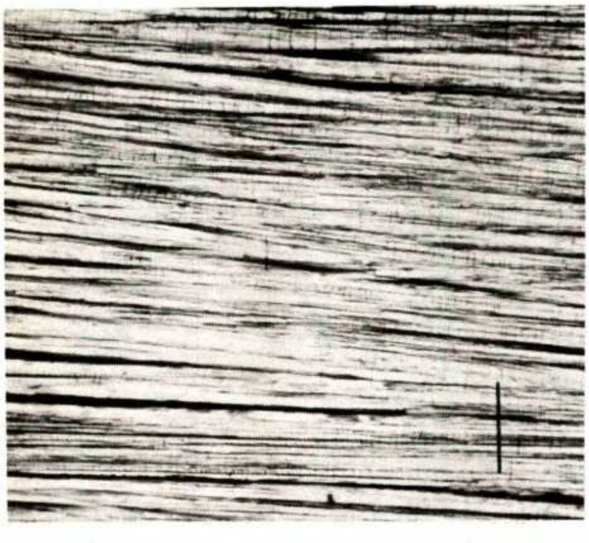

Fig. 2 Optical micrograph (transmitted light, crossed polars) of filament containing fluor-richterite and some pyroxene, drawn at 11 $\mu m\ s^{-1}$. Longitudinal section. Scale bar, 100 μm.

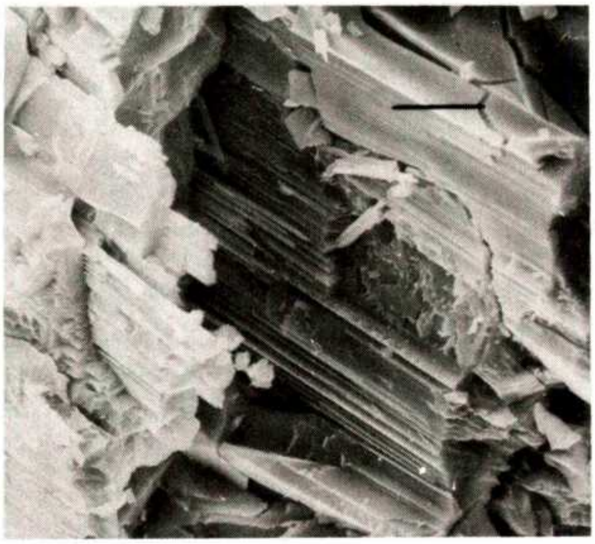

Fig. 3 Scanning electron micrograph of fracture surface of a filament containing fluor-richterite as the only crystalline phase. Scale bar, 10 μm.

reflections, correspond closely in intensity and position to the pattern given by a natural richterite from Langban, Sweden, supplied by the British Museum (Natural History) which matched the data reported by Huebner and Papike[8] for the synthetic richterite $Na_2CaMg_5Si_8O_{22}(OH)_2$.

Optical examination of thin sections of filaments, between 1 and 2 mm in diameter, reveal in most cases a well aligned, polycrystalline microstructure (Fig. 2) with fibres parallel to the filament axis. The high degree of alignment was confirmed by rotation X-ray diffraction. Crystal diameters vary according to conditions used but are rather large, being typically 10–20 μm, and in the case of fluor-richterite filaments free from pyroxene, larger than this (~20–60 μm). Transverse sections show that the fibre crystals are of pseudo-hexagonal or rhombic skeletal cross-section with cleavage planes at angles of approximately 56° and 124° as typical of amphiboles. The residual glass is estimated from transverse sections to be approximately 10%.

Figure 3 is a scanning electron micrograph of a typical fracture surface of a fluor-richterite filament showing the very much finer sub-structure to these crystals, made up of laths a fraction of a micrometre thick.

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Laser observations of the ground-state hyperfine structure of sodium and of temperatures in the upper atmosphere

A. J. Gibson, L. Thomas & S. K. Bhattachacharyya

SRC Appleton Laboratory, Ditton Park, Slough, Berks, UK

The height distributions of sodium between about 80 and 100 km have been measured by several groups from observations of the resonance scattering of a tuned laser beam[1–7]. Furthermore, estimates of temperatures in this height range have been made[4] by incorporating a sodium cell of known temperature in the receiver of the laser radar system. In all these measurements, the linewidths of the lasers employed have been comparable with, or greater than, that of the sodium D_1 or D_2 resonance line. The ground level of sodium atoms, 3s $^2S_{1/2}$ consists of two hyperfine-structure components at −1.107 GHz and 0.664 GHz relative to the centroid[8]. Because of the corresponding hyperfine structure of the upper levels, 3p $^2P_{1/2}$ for D_1 and 3p $^2P_{3/2}$ for D_2, four components contribute to the D_1 and six to the D_2 complexes. Since the relative strengths and frequencies of the ten components are well known, the theoretical line shape can be computed[9]. For temperatures above about 6 K, blending of these components occurs and for temperatures below about 456 K the line profiles of both D_1 and D_2 complexes show two distinct peaks. In this letter preliminary measurements of the profile of the D_2 line using a tunable narrow-band laser are presented and used to deduce the temperature near the peak of the sodium layer.

The effects of saturated absorption and optical pumping between the hyperfine levels of sodium atoms observed in laboratory measurements[10,11] are expected to be negligible with the moderate output energy (20 mJ) and the transmitter beam divergence (2.5 mrad) used.

The laser transmitter system described previously[12] has been modified by extending the resonator length and incorporating a piezo-electric etalon of 20 mm spacing to provide a tunable linewidth of about 0.1 GHz, about one-thirtieth of the widths of the D_1 or D_2 complexes at temperatures in the 80–100 km height range. The laser frequency, laser triggering, and data recording were controlled by a programable calculator, interfaced with a digital-to-analogue converter and with 10 range-gated photon counters. The laser frequency was changed, and the photon counts were transferred to the calculator, after every laser pulse. The system was programmed to perform successive scans each of eight equally-spaced laser frequencies spanning the D_2 line; after 75 scans comprising 600 laser pulses, occupying about 10 min, the data were recorded on magnetic tape.

The first results, based on 1,200 laser pulses during between 2300 and 2330 h on the night of 30 January 1979, are presented in Fig. 1*a*. This shows the ratio, R_i, of the signal from the sodium layer (82–98 km) to that from a lower height range (28–30 km), which is dominated by Rayleigh scattering, with the standard error, σ_i of this ratio for each frequency, $i = 1$ to 8. The variation of this ratio with frequency represents the corresponding variation of sodium atom cross-section. Also shown is the theoretical curve which gives the best fit to the data. Since an absolute calibration of the laser frequency was not available, this frequency was taken to be a linear function, $A+Bi$, of the frequency number, i, with two unknown parameters A and B. The theoretical curve is thus a function of four independent unknown parameters, that is

$$S_i(T,A,B,C) = C\sum_{h=1}^{6}\frac{f_h}{\nu_h\sqrt{T}}\exp\{-(A+Bi-\nu_h)^2Mc^2/2\nu_h^2kT\} \qquad (1)$$

where f_h and ν_h are the strength and frequency of the hth hyperfine component, M the mass of a sodium atom, c the velocity of light and k is Boltzmann's constant. The temperature T, and C, a scale factor for the ordinate in Fig. 1*a*, are the other two unknowns. The best fit of equation (1) to the data was obtained by minimising the χ^2 statistic given by

$$\chi^2 = \sum_{i=1}^{8}\{R_i - S_i(T,A,B,C)\}^2/\sigma_i^2 \qquad (2)$$

A test of the hypothesis that each of the observed values of R_i is a random sample from a normal distribution having mean $S_i(T,A,B,C)$ and variance σ_i^2, can be applied by determining the probability, $P(\chi^2)$, of the value of χ^2 obtained being exceeded in random sampling. The procedure for determining the means involves four nonlinear constraints, the equations

$$\frac{\partial(\chi^2)}{\partial q} = 0 \quad \text{where } q \equiv T, A, B \text{ or } C \qquad (3)$$

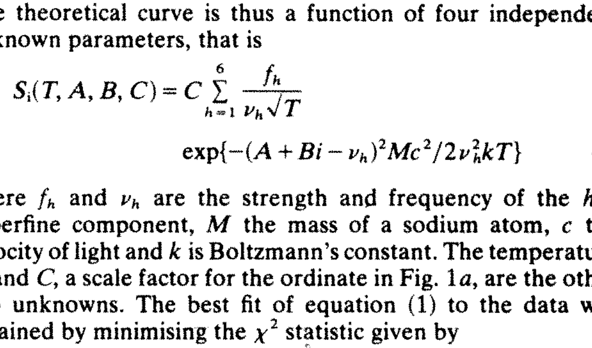

Fig. 1 *a*, Ratio of the sodium to Rayleigh scattered signals as a function of laser frequency for 1,200 laser shots on 30 January 1979, together with the theoretical lineshape which provides the best fit to the data. *b*, The probability $P(\chi^2, 4)$ as a function of temperature for the curve-fitting in *a*.

and hence we take the number of degrees of freedom as 4, obtaining a value $P(\chi^2, 4)$ of 0.67, indicating that the data are consistent with the theoretical relation (1).

Figure 1*b* shows the value of $P(\chi^2, 4)$ plotted as a function of T after χ^2 has been minimised with respect to A, B and C. The best value of T, indicated by the maximum of this curve, is (222±16) K; the error estimate corresponds to one-half the half-width of the P curve at $P=0.05$. Subsequent measurements during May 1979 indicated substantially lower temperatures, near 170 K, between 90 and 94 km. Small negative corrections can be applied to the measured temperatures after taking into account the finite laser linewidth. If $\Delta\nu_{\mathrm{D}}$ represents the Doppler linewidth of each hyperfine component and $\Delta\nu_{\mathrm{L}}$ is the laser linewidth, it can be shown by replacing equation (1) by its convolution with a gaussian laser line-shape function that the first-order correction is given by

$$\frac{\Delta T}{T} = -(\Delta\nu_{\mathrm{L}}/\Delta\nu_{\mathrm{D}})^2 \tag{4}$$

which amounts to less than 3K for our measurements.

It is to be expected that the present method of measuring upper atmosphere temperatures will be capable of greater accuracy than that involving the use of a sodium absorption cell in the receiver which depends on integration over the spectral line shape. The accuracy currently available with the new technique can be substantially improved by the use of high laser repetition rates, improvements in receiver sensitivity and maximum photon counting rates, and optimum choice of laser frequencies. The capability of observing the small-scale structure of temperature offered by this laser technique is directly relevant to studies of the thermal balance of the upper mesosphere, of the neutral and ion chemistry involving temperature dependent rate coefficients, of noctilucent clouds and of short-wavelength atmospheric-wave disturbances. This ground-based technique could, therefore, be a useful supplement to existing satellite measurements of temperature based on observations of thermal emissions.

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Environmental fluctuation effects on the global energy balance

C. Nicolis

Institut d'Aéronomie Spatiale de Belgique, 1180 Bruxelles, Belgique

G. Nicolis

Faculté des Sciences de l'Université Libre de Bruxelles, Campus plaine, CP 226, 1050 Bruxelles, Belgique

Much effort has been devoted to developing simple energy-balance climatic models. Although consideration of latitudinal energy transfer[1–4] gives more complete answers it has become clear that global, 'zero dimensional' models may also provide much useful information[5,6]. These models have the form:

$$C\frac{\mathrm{d}T}{\mathrm{d}t} = Q(1-a(T)) - \varepsilon\sigma T^4 \tag{1}$$

T **is the surface temperature,** ***C*** **the thermal inertia coefficient,** ***Q*** **the solar constants,** ***σ*** **the Stefan constant,** ***a*(*T*)** **the (generally temperature-dependent) albedo, and** ***ε*** **the emissivity of the Earth–atmosphere system. The variability of the climate system rests, therefore, on certain types of change experienced by the solar output, or by such planetary factors as emissivity, albedo, cloudiness and so forth. In addition to some long-term trends of the solar constant[7], it has been suggested that the Sun is in an almost-intransitive state[8,9]. Hence, it may generate large fluctuations around some mean value of its output, which will be perceived by the Earth–atmosphere system as an 'external noise' affecting** ***Q*****. The fact that the terrestrial atmosphere is likely to be in an almost intransitive state[10] can also generate appreciable fluctuations in factors influencing the albedo and the emissivity. In the absence of precise knowledge of the mechanism of these fluctuations, one is again tempted to regard them as an 'external noise' affecting** ***a*(*T*)** **and** ***ε*****. We explore here the qualitative effect of such environmental fluctuations in the thermal regime[13], at the level of a zero-dimensional planetary model. Previous analyses of nonlinear systems of chemical and biological interest[11,12] have shown that external noise can dramatically affect the macroscopic behaviour predicted by the deterministic equations of evolution, if coupled to these equations in a multiplicative way.**

All cases considered here refer to such multiplicative coupling. In this respect, our analysis differs from the work of Lemke[14] and Fraedrich[5] which is concerned primarily with additive fluctuations. Although such fluctuations are important, clearly the multiplicative coupling is a more appropriate representation of the dynamics of a complex system such as the Earth–atmosphere.

From the mathematical point of view, the effects of external noise change equation (1) to a stochastic differential equation, to which the Itô calculus can be applied[15]. Let us adopt an abstract notation and decompose equation (1) into two pieces, $f(T)$ and $g(T)$, which are linearly coupled through some parameter λ

$$\frac{\mathrm{d}T}{\mathrm{d}t} = f(T) + \lambda g(T) \tag{2}$$

Let λ be subject to environmental fluctuations referred to above. For simplicity, we take the idealised picture of a gaussian white noise. (The possible shortcomings of white noise, related to the possibility that the fluctuating parameters reach negative values, means that in each case the nature of the boundaries of the stochastic process must be checked carefully.) Hence[15]:

$$\lambda = \bar{\lambda}(1+\xi_t)$$

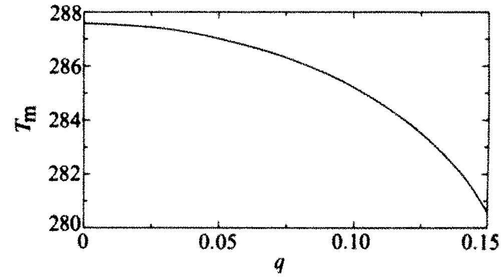

Fig. 1 Dependence of most probable temperature T_m on variance q. Q fluctuates around the mean value of 1.95 cal cm^{-2} min^{-1}. The thermal inertia coefficient used in the simulation is $C = 7.5$ kcal cm^{-2} K^{-1}. The emissivity ε is kept at the value 0.6, and the albedo is $a = \alpha + \beta T$ where $\alpha = 2.7888$, $\beta = -0.0086$.

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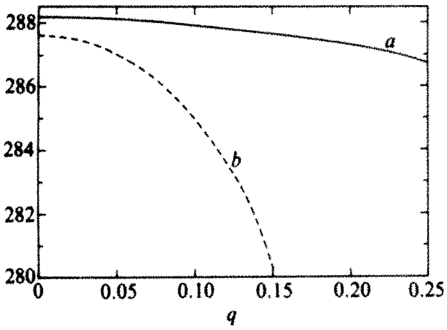

Fig. 2 T_m versus q when ε fluctuates around the value of 0.6. The thermal inertia coefficient is as in Fig. 1. *a*, The albedo is taken to be constant, $\bar{a} = 0.31$. *b*, The albedo is described by $a = \alpha + \beta T$ where $\alpha = 2.788$, $\beta = -0.0086$.

where $\bar{\lambda}$ is the value of λ appearing in the deterministic description and ξ_t is the derivative of a Wiener process, W_t:

$$\langle \xi_t \rangle = 0$$
$$\langle \xi_t \xi_{t'} \rangle = q^2\, \delta(t - t') \tag{3}$$

Equation (2) then takes the form (we now use the Itô rather than the Stratonovich interpretation[15]):

$$\mathrm{d}T = [f(T) + \bar{\lambda} g(T)]\,\mathrm{d}t + q\bar{\lambda} g(T)\,\mathrm{d}W_t \tag{4}$$

which is equivalent to a Fokker–Planck equation for the probability distribution $P(T, t)$:

$$\frac{\partial P}{\partial t} = -\frac{\partial}{\partial T}[f(T) + \bar{\lambda} g(T)]P(T, t) + $$
$$+\tfrac{1}{2} q^2 \frac{\partial^2}{\partial T^2} \bar{\lambda}^2 g^2(T) P(T, t) \tag{5}$$

A macroscopically observable stable temperature clearly corresponds to a maximum of the steady-state distribution $P_{st}(T)$ which obeys the equation:

$$-[f(T_m) + \bar{\lambda} g(T_m)] + \frac{q^2}{2} \frac{\mathrm{d}}{\mathrm{d}T}[\bar{\lambda}^2 g^2(T_m)] = 0 \tag{6}$$

we now apply this relation to the following situations.

(1) Q fluctuates around the present-day value of 1.95 cal cm^{-2} min^{-1}. If the planetary albedo, a, is taken to be constant, the noise becomes additive and equation (6) gives $\mathrm{d}g^2/\mathrm{d}T = 0$; hence one obtains the usual value of planetary temperature corresponding to present-day conditions. On the other hand, the fluctuations of Q begin to be effective if the planetary albedo depends on T. The simplest non-trivial such dependence is given by the linear function $a(T) = \alpha + \beta T$. Such expressions have been used widely[5,6,16], at least in the range between the highest temperature than can exist when the whole Earth is covered with ice, and the lowest possible temperature when no ice is present. Note that some pathological properties of linear albedo–temperature feedback pointed out by Schneider and Gal-Chen for one-dimensional energy-balance models[17] are not necessarily reproduced in a zero-dimensional planetary model. In the latter, the albedo must depend on the planetary temperature in a continuous fashion at least in a range of temperatures not too remote from present-day conditions.

(2) ε fluctuates around some mean value $\bar{\varepsilon}$ corresponding to a surface temperature of 287.6 K in the absence of fluctuations. The planetary albedo is taken to be, successively, equal to a constant value $\bar{a}$ or to a linear function of T. Physically speaking, fluctuations of ε at fixed albedo could be attributed to random modifications of atmospheric composition.

(3) a and ε both follow the fluctuations of cloudiness, n. The parameterisations of a and ε in terms of n are taken from Cess[16] and Schneider[18], and

$$n = 0.5(1 + \xi_t) \tag{7}$$

We now describe the results of some representative numerical simulations.

Figure 1 shows T_m as a function of q, the square root of the variance of Q. As q increases from 0.005 to 0.05 the most probable temperature decreases from about 287.6 K to 287 K. For higher q the decrease of T_m is more pronounced.

Finally, when $q \geq 0.16$ then T_m drops to extremely low values. The precise numbers are of no importance here, as the model should not be valid anyway for such ranges of temperature and additional feedback mechanisms could take over. The main point therefore of Fig. 1 is the tendency for runaway to lower temperature values.

Curve *b* of Figure 2 shows the analogous result in terms of the variance of ε for a linear albedo–temperature feedback (note that in this case equation (6) is an equation of seventh degree in T_m—a degree higher than that of the deterministic equation). Again, for $q \geq 0.16$ there is a similar tendency of runaway to extremely low values of T_m. Curve *a* describes the situation in the constant albedo case: the same tendency to lower T is observed, although there seems to be no runaway effect.

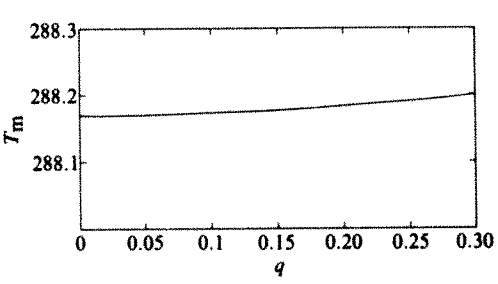

Fig. 3 Effect of fluctuating cloudiness using the following parameterisations. For the albedo (see ref. 16): $a = 0.18 + 0.26n$. For the emissivity (see ref. 18, for an effective cloud height of about 6.5 km): $\varepsilon = 0.71 - 0.22n$.

The effect of fluctuating cloudiness is rather different and described in Fig. 3 (here again, the equation for T is of seventh degree). As q increases from 0 to 0.30, the temperature remains substantially the same, varying by less than one-tenth of a degree. It appears that the fluctuations have opposite effects on the input and output terms in equation (4), thus giving a small overall effect. Another cause of the smallness of the effect may be that in the parameterisation of equation (7), cloudiness is taken to be temperature independent.

The thermal regime in a fluctuating environment seems thus to be established at temperatures that may differ substantially from those corresponding to a constant environment (actually, there always seems to be a tendency towards decreasing values). Eventually, in the presence of giant fluctuations a runaway regime may be reached in which there cannot be a stationary solution in a physically reasonable temperature range. These unexpected phenomena are all due to the multiplicative coupling between fluctuating parameters and the temperature[11]. In the case of additive noise or of linearised response[5,14] the effect would vanish because the function g^2 in equation (6) would be constant. One would thus recover the solution of the deterministic rate equation. It would be interesting to extend the present investigation to more realistic types of noise and to conditions prevailing in other planets, where a runaway regime is believed to exist.

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Intercalation of amino acids and polypeptides into 2H-TaS$_2$

V. M. Chapela* & G. S. Parry

Department of Chemical Engineering and Chemical Technology,
Imperial College, London SW7, UK

2H-TaS$_2$ can be intercalated by organic amines and empirical rules have been formulated[1–3] governing the properties that chemical species must possess in order to intercalate. One of these rules is that molecules in which the nitrogen is basic (pKa$>$4) usually form stable compounds provided the amine does not have bulky substituents. For certain amino acids, the values of pKa are significantly larger than this critical value (for example, glycine $pK_2 = 9.78$)[5] so that if the zwitterion is in a state where it can function as a base, it might be expected that the amino acid would intercalate 2H-TaS$_2$. This prediction has been verified for a number of amino acids. The discovery of these intercalation reactions provides a new range of intercalated compounds for study and also opens up the possibility of *in situ* polymerisation.

Saturated solutions of amino acids (chromatographically homogeneous or laboratory grade purity) in distilled water were prepared in a borosilicate glass ampoule at 75 °C. Samples of about 0.15 g of 2H-TaS$_2$, in either powder or single crystal form, were added and the ampoule was then sealed in air. The tubes were placed in an oven at 100 °C for between 1 and 3 weeks.

The intercalation reaction was monitored by opening capsules sequentially and examining the powder on an X-ray diffractometer with CuKα radiation. When the reaction product was entirely the stage 1 compound (judged by the position and line breadth of the 00l reflections), the new interlayer separation was obtained by extrapolating the parameter estimated from individual 00l reflections to $\theta = 90°$ as a function of cot θ cos θ. Selected single crystals were also examined using a single crystal goniometer with MoKα radiation.

The composition x in the formula TaS$_2$ (amino acid)$_x$ was determined using a Perkin-Elmer thermobalance. In making this calculation, the entire weight loss was attributed to amino acid for there was no sudden change in the rate of weight loss as might be expected if water was also intercalated along with the amino acid.

Superconducting transition temperatures were measured using a lock-in variable impedance method. Temperatures were measured using an Allen-Bradley carbon resistance thermometer calibrated against the vapour pressure of liquid helium and the superconducting transition temperatures of lead and niobium as calibration points. As noted in Table 1, certain compounds did not exhibit a superconducting transition between 1.2 and 15 K.

The intercalated species are shown in Table 1 and include amino acids such as glycine, β-alanine, L-proline, picolinic acid, diketopiperazine (glycine anhydride) as well as the polypeptides of glycine to pentaglycine. Attempts to intercalate isonicotinic acid, DL-proline and L-hydroxyproline were not successful. Values of the expanded gap (the difference between the layer separation before and after intercalation) show that, except for valine, the molecules lie parallel to the TaS$_2$ layer with the p$_z$ orbital containing the nitrogen lone pair orientated perpendicular to the layer. The results indicate that the peptide bond actively promotes intercalation. Thus glycine anhydride does not possess any amino groups at all and intercalation must have involved the two ring nitrogen atoms. Moreover pentaglycine (a 16-atom chain excluding hydrogens) remains parallel to the layer with an expanded gap of 3.4 Å in contrast to pentadecylamine[1] (also a 16-atom chain) which lies perpendicular to the layer with a 39 Å expanded gap.

Single-crystal X-ray studies indicate that the a-axis orientation of the matrix crystal is preserved on intercalation although the layer stacking order is destroyed (only hk rods are observed). This suggests a random sequence of stacking faults in contrast to the regular sequence of faults found in 2H-TaS$_2$(pyridine)[4]. Possibly the present choice of reaction conditions does not favour a highly orientated reaction product.

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* Permanent address: Centro de Investigacion de Materiales, UNAM, Apartado Postal 70–360, Cuidad Universiaria, Mexico 20, D.F., Mexico.

Table 1 Amino acids and polypeptides intercalating with 2H-TaS$_2$

Formula	Substance	pK_2*	Aq. conc. (g ml^{-1})†	x‡	Interlayer § separation (Å)		Expanded gap (Å)¶	Superconductivity T onset (K)	Superconductivity Width (K)
NH$_2$.CH$_2$COOH	Glycine	9.78	0.25	0.289	9.71	(0.009)	3.67	3.4	1.1
NH$_2$.(CH$_2$.CO.NH)$_{n-1}$.CH$_2$.COOH	Diglycine $n = 2$	8.25	0.125	0.172	9.43	(0.016)	3.39	2.6	0.6
	Triglycine $n = 3$	8.09	0.125	0.085	9.40	(0.017)	3.36	4.2	1.4
	Tetraglycine $n = 4$		0.036	0.076	9.4	(0.04)	3.4	No transition detected between 1.2 and 1.5 K	
	Pentaglycine $n = 5$		0.005	0.066	9.4	(0.04)	3.4		
CO.NH.CH$_2$.CO.NH.CH$_2$	Glycine anhydride (dikentopiperazine)		0.080	0.158	9.5	(0.05)	3.5	2.8	0.8
CH.CH.CH.CH.N.C.COOH	Picolinic acid		0.200	0.163	9.4	(0.03)	3.4	3.4	1.4
CH.CH.CH.N.CH.C.COOH	Nicotinic acid		0.166	0.165	9.56	(0.005)	3.52	3.6	1.8
CH(CH$_2$)$_2$.CH(NH$_2$).COOH	Valine	9.72	0.100	0.255	12.6	(0.07)	6.6	No transition detected between 1.2 and 15 K	
NH$_2$.CH$_2$.CH$_2$.COOH	β-Alanine		0.050	0.219	9.4	(0.04)	3.4	2.5	0.5
CH$_2$.CH$_2$.CH$_2$.NH.CH.COOH	L-Proline		0.400	0.181	10.00	(0.014)	3.96	4.7	2.7

* From ref. 5.
† Aqueous concentration: this solution was allowed to react for 7 days at 95 °C.
‡ Composition TaS$_2$(amino acid)$_x$.
§ The e.s.d. is given in parenthesis.
¶ The difference between the interlayer separation before and after intercalation.

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The superconducting transition temperatures are generally lower than for the amine intercalates[1] suggesting that the density of states at the Fermi level is modified less than in the amines. No superconducting transition could be detected in three of the compounds. The result was unexpected and further study is necessary before we can comment on the significance of this finding.

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Effect of *in situ* leaching on amino acid racemisation rates in fossil bone

Kenneth King, Jr

Lamont–Doherty Geological Observatory of Columbia University,
Palisades, New York 10964

Jeffrey L. Bada

Scripps Institution of Oceanography, and Institute of Marine Resources,
University of California, San Diego, La Jolla, California 92093

Amino acid racemisation reactions provide a promising approach for estimating the absolute ages of fossil bones[1]. However, an important question remains unanswered: what is the effect of *in situ* leaching on apparent racemisation rates[2,3]? We report here the relationship between leaching history and the corresponding D/L aspartic acid ratio for 12 well-dated fossil samples, using γ-carboxyglutamic acid (Gla) as a quantitative index of leaching[4], in an attempt to resolve this problem.

High-temperature simulation studies provided the initial evidence that intensive leaching of bone organic matrix can substantially change the D/L ratios of the matrix amino acids and consequently generate misleading apparent ages[2,3]. In contrast, racemisation ages of natural fossil samples (presumably representing a range of different leaching histories) were found to be in close agreement with independently determined radiocarbon dates[5–7]. Availability of a bone-specific leaching index (Gla) unaffected by environmental organic matter[4] has allowed us to study directly the possible role of leaching in the alteration of the D/L ratios used for the amino acid dating of fossil bones.

The six pairs of fossil bone samples in which Gla was measured included a wide range of ages, environmental temperatures, and leaching histories (Table 1). The first two samples (Buhen Horse and Mindanao SU-1) came from localities having nearly identical air temperatures. However, their respective environments were quite different in terms of annual rainfall—the Buhen Horse sample was from the arid Sudan ($0.4\ \mathrm{cm\ yr^{-1}}$ precipitation) and the Mindanao SU-1 sample was from an estuarine swamp in the Philippines ($232\ \mathrm{cm\ yr^{-1}}$ preciptiation)—and this is reflected by their Gla values of 845 and $516\ \mathrm{nmol\ g^{-1}}$, respectively. As percentages of the Gla concentration in fresh bovine bone (1,076 nmol per g bone[4]), the Gla content of the Buhen Horse sample is 79% and the Mindanao sample 48%. Both samples have the collagenous organic matrices expected in slightly to moderately leached specimens[2]. Despite a significant difference in leaching histories reflected by the Gla contents, the two samples show essentially equal racemisation rate constants (k_{asp}) when the environmental temperatures are taken into account.

Both of the La Jolla palaeo-Indian samples have undergone substantial leaching as shown by their low Gla values. Although sample SDM 16709 has a Gla content equivalent to only 5% of fresh bovine, its organic matrix is still collagenous in composition. When the D/L ratio of SDM 16709 is used to calibrate the racemisation reaction, the resulting racemisation age for W–12/76 is in good agreement with the radiocarbon date.

The sites at which the Olduvai Gorge and Nasera Rock Shelter samples were collected are only about 30 km apart. Both have been heavily leached, although the less intensely leached Olduvai Gorge sample (13% Gla content relative to fresh bovine bone) still has a collagen-like pattern. The calculated racemisation age of the Nasera Rock sample (using the Olduvai Gorge specimen for calibration) is within 8% of its radiocarbon date.

The Murray Springs and Double Adobe samples, very similar in age, are also intensively leached as shown by the Gla contents and the absence of collagen in both organic matrices. As a result of good agreement between the D/L aspartic acid ratios of the Double Adobe sample and the calibrating sample (Murray Springs), the calculated racemisation age is concordant with the radiocarbon age.

The two older samples from the Nasera Rock Shelter (levels 5A and 6) have similar radiocarbon ages. Both have undergone severe leaching and neither show detectable Gla nor a collagenous organic matrix. The racemisation age of level 6, based on a calibration with level 5A, is about 13% higher than the radiocarbon age.

The oldest pair of samples is represented by two from Mt Carmel. Both are leached to an extreme degree as shown by the absence of Gla in the Tabūn sample (non-collagenous) and the trace of Gla in the Sefunim specimen (collagenous). Although there is an age difference of ~14,000 yr between the Sefunim calibration sample and the Tabūn sample, the calculated racemisation age for the Tabūn specimen is within 8% of the radiocarbon age.

The data in Table 1 indicates concordance of aspartic acid racemisation age with radiocarbon data for each of the six unknown samples (despite measurable differences in leaching history between the unknown and calibration samples). Thus, *in situ* leaching seems to have a negligible effect on the racemisation parameters calculated from these six pairs of fossil bone samples.

How can this finding be reconciled with the original laboratory results showing significant alteration of racemisation rates with intensive leaching[2,3]? A closer examination of the

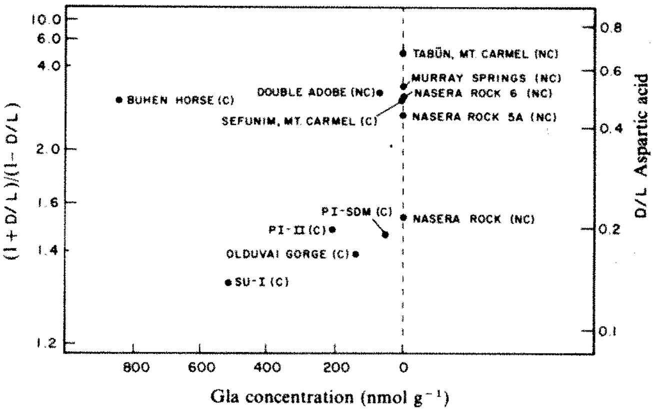

Fig. 1 Relationship between aspartic acid racemisation parameters and degree of *in situ* leaching. Intensity of leaching increases with decreasing values of Gla content. The parameter $\ln[(1+\mathrm{D/L})/(1-\mathrm{D/L})]$ is the key variable in the equation used to calculate racemisation ages[8]. C, collagenous matrix; NC, non-collagenous matrix.

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leaching data (Fig. 1) suggests an answer to this question and helps to define the conditions in which leaching could adversely affect racemisation calculations.

The 12 samples segregate into two major clusters (Fig. 1): a low aspartic acid D/L ratio group ($\bar{X} = 0.18$), and a high aspartic acid D/L ratio group ($\bar{X} = 0.51$). The lower cluster of five samples can be characterised as young in age ($\bar{X} = 4{,}100$ yr), moderately leached (only one sample has < 0.1 nmol Gla per g), and predominantly collagenous in composition (only one sample contains a non-collagenous organic matrix). In contrast, the other group of seven samples with higher D/L values has a mean age of 12,400 yr, generally exhibits intense leaching (five samples have undetectable or trace amounts of Gla) and contains predominantly non-collagenous matrices (only two samples display a collagen pattern).

Note that amino acid concentration (Table 1), another possible leaching index, generates a plot remarkably similar to the Gla plot (Fig. 1). With the exception of the Buhen Horse/Mindanao comparison, the sample having the higher amino acid concentration in each pair also had the higher Gla content. However, the Mindanao sample, with a 28% higher amino acid concentration than the Buhen Horse specimen, showed a 39% lower Gla content. This unusual relationship is thought to result from the interaction of two factors: (1) minimal solubilisation (through decreased chain length) of the collagen in the Mindanao sample during the short time interval represented (~500 yr); and (2) a more rapid loss of Gla in the Mindanao sample due to the intensive leaching conditions of its estuarine environment. Although more susceptible to perturbation by environmental contamination, amino acid concentration could be used as a leaching parameter in very heavily leached samples after Gla has disappeared.

It is probably significant that, with only one exception (the slightly leached Buhen Horse specimen), the sample used to calibrate the racemisation reaction is in the same group as the unknown sample to be dated. This suggests the possibility that each of the calibration samples has, in effect, calibrated the racemisation reaction in the corresponding unknown for degree of leaching as well as environmental temperature. This hypothesis may be tested by attempting to 'cross-calibrate' between the two groups using data from the Nasera Rock Shelter site (Table 1). If the k_{asp} value ($7.4 \times 10^{-5}\ \mathrm{yr}^{-1}$) used for the young (~2,000 yr BP) Nasera Rock Shelter sample (low D/L group) is used to calibrate the racemisation process for dating the Nasera Rock Shelter Level 6 sample (high D/L group), the resulting age estimate is 6,200 yr BP or 73% lower than the radiocarbon age. In addition, if the k_{asp} value of the calibration sample (postglacial exposure) is reduced by 40% to compensate for the lower integrated temperature of the older Nasera Rock Shelter (Level 6) sample (postglacial and glacial exposures)[9], the calculated amino acid racemisation age is only increased to 10,400 yr BP (55% less than the radiocarbon age). As shown in Table 1, the

Table 1 Effect of *in situ* leaching on amino acid parameters in fossil bones

Site and description	Mean annual air temperature	D/L Aspartic acid*	k_{asp} (per yr)	Racemisation age (yr)†	Radiocarbon (yr) or historical age	Gla content (nmol g^{-1})‡	Amino acid concentration (nmol g^{-1})§	Organic matrix¶
Wadi Halfa, Sudan Buhen horse	26°C	0.474	1.21×10^{-4}	—	1,675 BC	845(11.8)	1,192,000	C
Mindanao, Philippines	27°C	0.136	1.48×10^{-4}	—	AD 15 –16th century	516(11.7)	1,522,000	C
SU-1 (Butnan City) Near La Jolla California Palaeo-Indian skeletons								
SDM 16709 (PI-SDM)	16°C	0.189	1.45×10^{-5}	—	8,360±75 (Pta-1712)	51(0.6)	81,150	C
W-12/76 No. II (PI-II)	16°C	0.195	—	8,800	8,330±160 (Pta-1809)	211(3.9)	381,600	C
Olduvai Gorge, Tanzania	25°C	0.165	7.4×10^{-5}	–	1,390±40 (LJ-2979) 1,330±100 (LJ-3330)	144(1.0)	424,400	C
Nasera Rock Shelter, Tanzania (~30 km from Olduvai)	25°C	0.212	—	1,960	2,060±100 (ISGS-438) 2,180±200 (ISGS-438)	<0.1	4,292	NC
Murray Springs, Arizona, nos 23–51	17°C	0.520	4.5×10^{-5}	—	11,230±340 (A-805)	<0.1	1,685	NC
Double Adobe, Arizona, nos 15–45	17°C	0.500	—	10,600	10,420±100 (A-1152)	70(1.2)	3,103	NC
Nasera Rock Shelter, Tanzania								
Level 5A	25°C	0.428	1.81×10^{-5}	—	21,600±400 (ISGS-445)	<0.1	1,814	NC
Level 6	25°C	0.486	—	26,000	22,900±400 (ISGS-425)	<0.1	1,182	NC
Mt. Carmel, Israel Sefunim layer 8–9	19°C	0.472	1.64×10^{-5}	—	~27,000 Upper Paleolithic	4(0.4)	7,344	C
Mt Carmel, Israel Tabūn lower layer C	19°C	0.658	—	44,000	40,900±1,000 (GrN-2729)	<0.1	1,430	NC

* All samples hydrolysed with 6M HCl for 24 h D/L. Aspartic acid ratios were determined as described elsewhere[7,8].

† Calculated by using the k_{asp} of the other pair member as the calibration sample as previously described[7].

‡ Mean values and standard deviations (in parentheses) for duplicate analyses of single fossil samples. Alkaline hydrolysis and Gla analysis of each 2-mg sample were conducted as previously described[4]. The Gla content of fresh bovine cortical bone is about 1,100 nmol g^{-1} (ref. 4).

§ Summation of 12 individual amino acid concentrations (Asp, Thr, Ser, Glu, Gly, Ala, Val, allo-Ileu, Ileu, Leu, Tyr, Phe).

¶ C, collagenous; NC, non-collagenous. Determined by amino acid analysis of acid hydrolysates.

racemisation age for this same sample calibrated with the Nasera Rock Shelter Level 5A sample (same high D/L group) is 26,000 yr BP, 13% higher than the radiocarbon date. This example further suggests that a 'cross-over' between the two groups in calibrating a racemisation reaction may introduce substantial error in estimating absolute age.

The need for intra-group calibration most probably stems from key compositional differences between the two groups produced, in part at least, by *in situ* leaching. Although detailed information on the relevant properties (for example, molecular weight, amino acid composition, and degree of racemisation) of each individual matrix component is not yet available, comparative total amino acid compositions provide some evidence for a requirement of compositional similarity between calibration and unknown samples. As shown in Table 1, four of the six racemisation calculations involved sample pairs having organic matrices of similar composition (2 collagenous; two non-collagenous). Note that the only successful cross-calibration between the two groups involves one of the compositionally similar pairs—the collagenous Buhen Horse and Mindanao SU-1 samples. These two samples also show the lowest degrees of leaching among the samples analysed. Successful calibration with pairs of similar organic matrices is not unexpected as the laboratory leaching data indicates changes in racemisation rates coincident with the compositional change from collagenous to non-collagenous matrix[10].

The remaining two pairs each involved a combination of different matrices—Olduvai Gorge (collagenous)/Nasera Rock Shelter (non-collagenous) and Sefunim (collagenous)/Tabūn (non-collagenous). An explanation for successfully achieving concordancy with these unlike matrices will require detailed characterisation of the major diagenetic components of each matrix to reveal key compositional and stereoisomeric similarities not evident in overall amino acid composition. Nevertheless, it is interesting that the calibration and unknown samples of each pair are in the same D/L group (Fig. 1) despite their dissimilar compositions.

No significant alteration in racemisation rates by *in situ* leaching was detected in this study of six pairs of fossil bone samples. The data (Fig. 1), however, suggest that the general concordancy observed between racemisation ages and radiocarbon dates may have resulted from a close matching of the calibration and unknown samples in each pair in terms of composition, leaching history and degree of racemisation. Accordingly, we recommend that amino acid composition, Gla content and amino acid concentration be measured with the D/L ratio in both the calibration and unknown samples to minimise the possibility of introducing significant errors in the calibration step. In addition to improving the reliability of the racemisation dating method, the routine measurement and reporting of these parameters would, over a period of time, generate a valuable data base for profiling the leaching–racemisation relationship at ambient temperatures.

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Origin and palaeoenvironmental interpretation of sarsens

M. A. Summerfield

School of Geography, Mansfield Road, Oxford, UK

No detailed explanation has yet been provided for the origin of sarsens (silicified Cenozoic sediments) which are widely distributed across southern England (Fig. 1). The problem of sarsen formation is related to that of analogous terrestrial siliceous deposits recorded from every continent except Antarctica[1–7]. In addition sarsens are of considerable archaeological interest as they were extensively used in the construction of megalithic monuments, notably those at Avebury and Stonehenge. It has been suggested that sarsens are glacially transported erratics[8,9], although this view has been challenged[10]. Their occurrence in areas in which other evidence of glacial activity is lacking, and the preservation on the surface of some sarsen stones of pockets of very weakly cemented sand, does not concur with the idea of long-distance transportation, but rather suggests local derivation from an original Cenozoic cover. Here the initial formation of sarsens is considered and recognition that they represent silcrete remnants enables broad conclusions to be drawn about their genesis and palaeoenvironmental significance. From macromorphological, micromorphological and chemical comparisons with low latitude silcretes, primarily from southern Africa, it is concluded that sarsens represent remnants of surface and near-surface silicification developed on tectonically stable landsurfaces of minimal local relief. Most sarsens seem to have formed under a semi-arid or arid climate, although there is evidence of development in a relatively humid environment for some occurrences.

As there are no entirely reliable reports of *in situ* sarsen occurrences, field evidence is confined to individual sarsen stones, up to 5 m or more across, found resting on the Chalk and on, or within, Cenozoic sediments. Sarsens range from orthoquartzites to predominantly fine-grained siliceous sediments and silicified conglomerates (Hertfordshire and Bradenham Puddingstones). A surface, or near-surface, origin is indicated by the presence of apparent rootlet channels in many samples and, more rarely, by silicified roots[11]. Sarsens represent various forms of silcrete[12] and consequently their genesis may be elucidated by comparison with the extensive silcrete occurrences of Africa and Australia.

Sarsens are highly siliceous sediments (Table 1). Micromorphologically they can be classified into GS-(grain-supported), F-(floating) and C-(conglomeratic) fabric types[13]. GS-fabric sarsens consist of skeletal framework grains ($>30\ \mu m$ across), almost exclusively of quartz, usually cemented by optically continuous overgrowths, and less frequently by microquartz. F-fabric sarsens are composed of predominantly quartz skeletal grains, floating in a microquartz or cryptocrystalline silica matrix. C-fabric sarsens most commonly consist of flint pebbles in a 'matrix' of the GS-fabric type.

Field investigations of silcretes from southern Africa have shown that a distinction can be made between those occurrences associated with weathering profiles (weathering profile silcretes, WPS), and those that are not (non-weathering profile silcretes, N-WPS). These differences in field occurrence are accompanied by contrasting chemical and micromorphological characteristics. GS-fabrics, C-fabrics, optically continuous overgrowths, chalcedonic overgrowths and chalcedony vugh-fills are absent in WPS while M-(matrix) fabrics ($<5\%$ skeletal grains ($>30\ \mu m$ across)) are abundant (Table 2). In N-WPS authigenic glaebules and colloform features are apparently absent and M-fabrics are rare. WPS are titanium-rich ($TiO_2 > 1\%$) while N-WPS are titanium-poor ($TiO_2 < 0.2\%$) (Table 1). N-WPS are typically

Table 1 Bulk chemical analyses of sarsens and southern African silcretes

	SiO_2	Al_2O_3	TiO_2	Fe_2O_3	MgO	CaO	K_2O	MnO	P_2O_5	Ignition loss
Mean composition of 51 WPS from southern Africa	94.70	0.71	1.82	1.34	0.13	0.10	0.02	0.01	0.04	1.16
Mean composition of 13 N-WPS from southern Africa	93.92	1.43	0.13	0.66	0.55	0.12	0.68	0.07	0.03	2.41
GS-fabric sarsen with optically continuous overgrowths (A)	99.56	0.22	0.07	<0.01	—	<0.01	—	<0.01	<0.01	0.14
GS-fabric sarsen with optically continuous overgrowths (B)	98.79	0.15	0.03	—	0.47	0.02	—	0.01	—	0.54
F-fabric sarsen (C)	96.12	0.29	2.08	0.80	—	0.03	—	<0.01	0.02	0.66
C-fabric sarsen (D)	98.56	0.20	0.16	0.07	—	0.02	—	<0.01	0.02	0.96
C-fabric sarsen (E)	97.90	0.24	0.02	0.54	0.37	0.02	—	<0.01	0.04	0.88

Analyses by XRF normalised to 100%. The high mean K_2O content of N-WPS is associated with (glauconite-rich?) green pan silcretes (silicified pan clays). Letters in parentheses refer to sample locations in Fig. 1.

more chemically and micromorphologically variable than WPS as they represent the silicification of a wide variety of host materials including calcretes, fluvial deposits and pan sediments.

In the sarsen samples examined the GS-fabric type (with optically continuous overgrowths) is the most common. F-fabrics are relatively scarce, except locally. Comparative micromorphological evidence from southern African silcretes militates against a weathering profile origin for GS- and C-fabric sarsens (Table 2). The status of F-fabric sarsens is less certain in that the micromorphological and chemical evidence points to a weathering profile origin for at least some occurrences. This is supported by the high titanium content (2.08% TiO_2) (Table 1) of an F-fabric sarsen which is micromorphologically identical to many F-fabric WPS from southern Africa (Fig. 2). The titanium appears to be present in dull brownish aggregates (identified by X-ray diffraction as anatase in southern African silcretes) within the matrix. Titanium- and iron-rich zones as colloform features have also been reported in F-fabric sarsens (K. Isaac, personal communication). Further analyses are required to establish whether F-fabric sarsens are characteristically titanium-rich.

The nature of the host material and of the circulating solutions responsible for silicification are the key factors in influencing sarsen micromorphology. GS-fabric sarsens formed through passive void-filling by overgrowths or microquartz of a quartzose host sediment. The development of optically continuous overgrowths implies a lack of clay within the host sediment[14,15] and circulating solutions characterised by both low concentrations of silica and of other ions (which could disrupt the orderly growth of macro-crystals). Conversely the presence of intergranular clay and of relatively high concentrations of silica and/or 'disruptive' ions would promote microquartz or even cryptocrystalline silica precipitation[16]. A similar interpretation may be applied to C-fabric sarsens as these differ from the GS-fabric type only in the size range of their skeletal grain fraction. F-fabrics can be generated by displacement or partial replacement of skeletal grains or by replacement of the matrix of an F-fabric host material such as clayey weathering deposit containing floating quartz grains. Matrix replacement is the favoured interpretation for sarsens as evidence of marked skeletal grain replacement is lacking and as displacive growth of authigenic quartz within voids is an untenable explanation of F-fabrics with a small skeletal grain component (Fig. 2) because of the considerable volume increase required. In silcretes from southern Africa there is no field evidence for the anticipated macroscale expansion which would be associated with F-fabrics generated by displacive crystal growth (such as there is in some cases for calcretes in the form of pseudoanticlines).

Previous explanations of sarsen formation have focused on macroscale climatic factors and have largely ignored geochemical conditions and micromorphological properties. A capillary rise mechanism has been favoured involving silica mobilisation and surface concentration (due to evaporation) in response to a seasonally dry climate[11]. However, only thin surface crusts (rather than the typically observed short-axis sarsen dimensions of $\sim$1 m) could form in this way as initial surficial silica precipitation would inhibit, and eventually prevent, the upward movement of solutions to the surface.

Comparison with silcretes from southern Africa and Australia

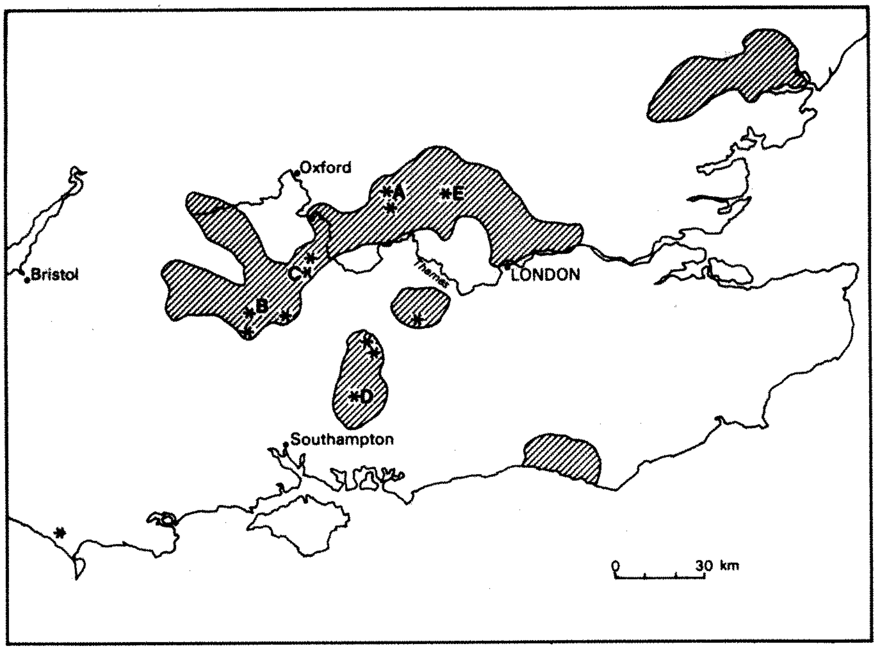

Fig. 1 Distribution of sarsens in southern England. Shaded areas indicate main regions of sarsen concentrations. Sample sites marked by asterisk. For key to letters see Table 1.

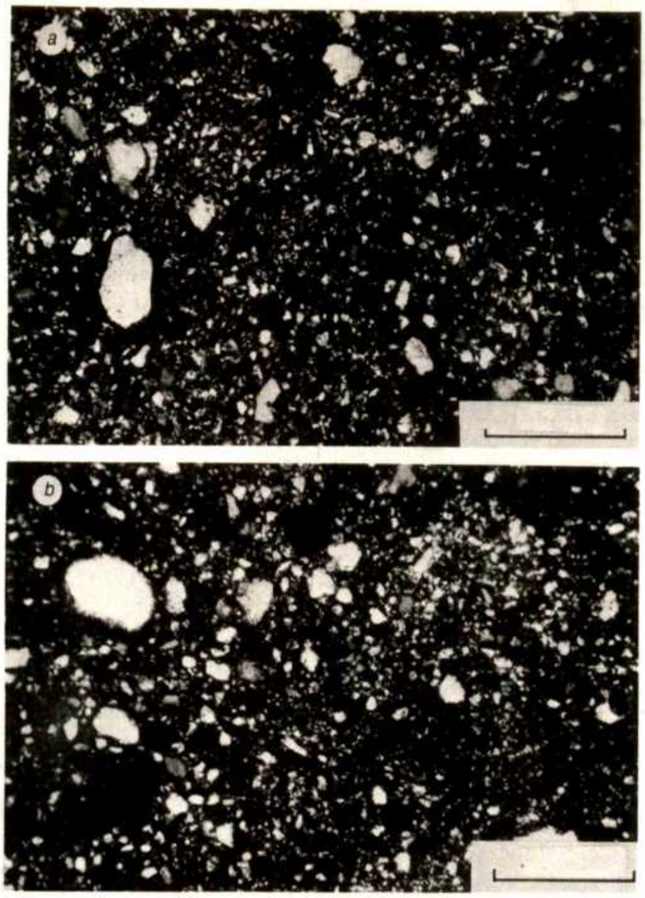

Fig. 2 Photomicrographs of *a*, F-fabric sarsen (sample C in Table 1) and *b*, F-fabric silcrete from Riversdale, South Africa. Both samples have a small skeletal grain fraction and titanium-rich matrix (2.08% TiO_2 in *a*, and 1.72% TiO_2 in *b*). Crossed polarisers. Scale bars, 500 μm.

suggests several possible origins for sarsens. Probable local silica sources for N-WPS include direct dissolution of quartz grains and enhanced solubility quartz particles produced through grain abrasion, and release of silica during calcrete formation or from diatoms and other silica-secreting organisms. Silica may also be supplied from surface waters and atmospheric fall-out. A decrease in *p*H from >9 to <9, is considered to be a probable silica precipitating mechanism. Periodic changes in *p*H produced by dilution by fresh surface waters could produce alternating phases of high silica mobility (*p*H >9) and silica precipitation (*p*H <9). Alternatively, in the absence of *p*H changes, silica may gradually precipitate (particularly as overgrowths) from slowly migrating solutions only moderately supersaturated with respect to quartz. Water table fluctuations and downward percolation are regarded as the most viable models of N-WPS formation as these are the only mechanisms which allow the development of laterally extensive and relatively thick silcrete horizons.

Silica released within the weathering zone, either through bedrock weathering or clay mineral alteration (such as illite → kaolinite) provides the most likely silica source for WPS. Silica so released could become precipitated lower down the profile if drainage were sufficiently restricted. Aluminium mobilisation accompanying silica replacement of weathering profile clays would probably be associated with a low *p*H environment (*p*H <4) and titanium mobility and concentration in WPS supports this idea[13,17].

Table 2 Relative frequency of micromorphological features and fabrics in sarsens, WPS and N-WPS from southern Africa

Micromorphological feature/fabric	Sarsens	N-WPS	WPS
Optically continuous overgrowths	A	U	X
Chalcedonic overgrowths	X	U	X
Glaebules (authigenic)	X	X	A
Colloform features	U	X	A
M-fabric	X	R	A
F-fabric	U	A	A
GS-fabric	A	U	X
C-fabric	C	U	X

A, abundant; C, common; U, uncommon; R, rare; X, absent. No. of samples: sarsens, 18; N-WPS, 29; WPS, 93.

The proposed high *p*H environment and lack of associated deeply weathered sediments suggests a semi-arid or arid climate during N-WPS formation; a similar interpretation is appropriate for GS- and C-fabric sarsens. WPS probably formed under a relatively humid climate where rates of organic activity were sufficiently high to promote a low *p*H environment, and it is likely that titanium-rich F-fabric sarsens formed in similar conditions.

Future investigations of *in situ* sarsen equivalents in France (meulière) and elsewhere in mainland Europe associated with a more complete Cenozoic stratigraphic record should clarify the problem of dating sarsen formation and provide a basis for independent evidence for palaeoclimatic interpretation.

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Host specificity of temperate and tropical animals

R. A. Beaver

Department of Biology, University of Zambia, P.O. Box 2379, Lusaka, Zambia

Many groups of animals show a gradient of increasing species richness from polar to equatorial regions[1,2]. Theory[3,4] suggests that species should be more specialised ecologically in the tropics. Most of the evidence for this comes from studies on birds[5–10]. Birds appear to subdivide the habitat more finely and specialise more in their food in the tropics. It has been suggested[11] that tropical Papilionidae (Lepidoptera) are more specialised in their hosts than temperate species, but host specificity was considered only at the level of the plant family, and more detailed studies of a wide range of butterflies[12] suggest that they are little (if at all) more specialised in their food plants in the tropics. Similarly, monogenean trematode parasites of fish show no change in the degree of host specificity at different latitudes[13]. Here it is shown that one group of insects, the bark and ambrosia beetles (Coleoptera: Scolytidae and Platypodidae), are less host-specific in the tropics than in temperate regions, even though there are considerably more species present in the tropics. The 'reverse' latitudinal gradient in host specificity can be related to the greater proportion of relatively non-specific xylomycetophagous species (ambrosia beetles) in the tropics and to the greater heterogeneity of tropical forests which will make host specialisation more difficult.

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Table 1 Comparisons of area, latitude and number of species of Scolytidae and Platypodidae in some temperate and tropical countries

Country	Area (km^2)	Latitudinal range	Total no. of species	Expected no. of species*
France	549,600	43–51 °N	135	41.0–68.4
California	411,000	33–42° N	176	59.2–94.5
West Malaysia	131,300	1–6° N	512	256.9–345.2
Fiji	18,300	16–19° S	81	81

* No. of species expected in an area the size of Fiji, assuming a linear relationship between log (no. of species) and log (area) with a slope of 0.2–0.35.

Comparisons have been made between the bark and ambrosia beetle faunas of two temperate areas, France[14] and California[15], and two tropical areas, West Malaysia[16,17] and Fiji[18]. The data obtained from refs 14 and 16–18 have been extended and/or updated by reference to many other sources. Table 1 indicates a gradient in species richness from the temperate to the tropical zone when area is taken into account. The islands of Fiji are perhaps no richer in species than California if equal areas are compared, but relatively fewer species are expected in isolated oceanic islands than on the mainland.

The data are classified according to host specificity and feeding habits of the beetles in Table 2. (Terms used to describe the feeding habits are defined in Table 2 legend.) Information on both parameters is available for 98% of the species recorded in the temperate areas, but only 69% in Fiji and 54% in Malaysia. However, my own observations of the fauna of West Malaysia and of other tropical areas suggest that the main conclusions obtained here will not be significantly changed if further information becomes available for the tropical areas.

Errors are possible in the assignment of beetle species to different grades of host specificity. Thus if a host genus is monospecific in an area, or a host family is monogeneric, the true specificity of the beetle may be overestimated. Conversely, some species occasionally attack hosts outside their normal range. Breeding in such hosts may not occur, but the host may still appear in host lists. Errors in assignment are generally likely to overestimate host specificity and to be greater for the tropical areas. Corrections of such errors as more information becomes available will probably increase the observed differences between temperate and tropical areas.

Table 2 Number of species of Scolytidae and Platypodidae with different feeding habits confined to a particular species, genus or family of host plants or polyphagous

Country and feeding habits*	No. of species Species	Genus	Family	Polyphagous	Total
France					
Phloeophagous	2	60	43	1	106
Herbiphagous	1	5	2	1	9
Spermatophagous	0	0	2	0	2
Xylophagous	0	0	0	1	1
Xylomycetophagous	0	2	3	9	14
Total	3	67	50	12	132
% Of fauna	2	51	38	9	100
California					
Phloeophagous	14	96	34	3	147
Herbiphagous	0	2	1	1	4
Spermatophagous	6	1	1	0	8
Xylophagous	0	1	1	1	3
Xylomycetophagous	0	1	5	5	11
Total	20	101	42	10	173
% Of fauna	12	58	24	6	100
West Malaysia					
Phloeophagous	0	3	21	8	32
Herbiphagous	0	5	3	10	18
Spermatophagous	0	1	1	12	14
Xylophagous	0	0	1	0	1
Xylomycetophagous	0	1	72	137	210
Total	0	10	98	167	275
% Of fauna	0	4	35	61	100
Fiji					
Phloeophagous	1	6	3	7	17
Herbiphagous	0	0	0	4	4
Spermatophagous	0	1	0	2	3
Xylophagous	0	0	0	0	0
Xylomycetophagous	0	1	6	25	32
Total	1	8	9	38	56
% Of fauna	2	14	16	68	100

* Phloeophagous species construct gallery systems and breed in the phloem and inner bark of woody plants; herbiphagous species in the stems of non-woody plants, in the pith of twigs or in leafstalks; spermatophagous species in seeds and fruits. Xylophagous species live and feed on the wood; xylomycetophagous species usually live in the wood but cultivate and feed on ambrosia fungi growing on the walls of their galleries[19,20].

Table 3 Values of χ^{2*} based on percentages of species of Scolytidae and Platypodidae with different host specificity for pairs of countries

Species group	Countries compared† Fr/Ca	Fr/Ma	Fr/Fi	Ca/Ma	Ca/Fi	Ma/Fi
All	10.5	81.3	74.2	107.4	88.0	15.9
Phloeophagous	10.9	63.9	54.5	91.3	46.0	52.5
Xylomycetophagous	13.0	15.9	8.7	12.8	22.7	7.6

* Values greater than 7.8 are significant at the 5% level; 11.3 at the 1% level.

† Ca, California; Fi, Fiji; Fr, France; Ma, West Malaysia.

The Platypodidae (all xylomycetophagous) have been lumped together with the Scolytidae because (1) they are probably no more than a divergent subfamily of Scolytidae[21]; and (2) the proportions of Platypodidae in the different grades of host specificity in West Malaysia are not significantly different ($\chi^2 = 3.1$) from the proportions found in the xylomycetophagous Scolytidae in that country. The family is only abundant in West Malaysia among the study areas (63 species included in Table 2). It forms a minor part of the fauna of the other areas (one species in California, two in France, five in Fiji).

The results of χ^2 tests carried out on the percentages of phloeophagous and xylomycetophagous species and all species together are shown in Table 3. Numbers of species in the groups with other feeding habits are too low for comparisons to be made. Comparing the total fauna of the four areas, it is clear that the tropical beetles as a whole are far less host specific than those of temperate areas. There is far greater similarity within the tropical or temperate zones than between them. Host specificity is greatest in California and least in Malaysia. One reason for this is apparent from the more detailed analysis. A far higher percentage of the fauna consists of xylomycetophagous species in the tropics. This group shows low host specificity (Table 2). Values of χ^2 are relatively low for all comparisons within this group of species, indicating a similar low specificity in both temperate and tropical regions. The ambrosia fungi, on which both adults and larvae feed, will grow in a great variety of trees. It must be remembered that the beetle, so far as is known, feeds on the same fungi whatever host it is attacking, and it is really the fungi that are polyphagous and not the beetle. However, it is the beetle that selects the host tree.

The host specificity of the phloeophagous species is similar in France and California, and higher than in the tropical countries. The difference is perhaps related to the greater species diversity and heterogeneity of tropical forests. Specialisation is only possible if the resource(s) on which specialisation occurs is renewed in a way that is reasonably regular relative to the dispersive ability and lifespan of the species concerned. Too great a scattering of the resource in space or time and the species can not specialise on it[22]. The beetle–host tree system has two basic characteristics[23]. The first is that the habitat stability[24] of the habitat unit (plant stem, leafstalk, fruit or seed) is approximately unity, so that only one beetle generation is possible in the unit. The second is that habitat units in a suitable state for colonisation form a spatial patchwork in the environment. Since each unit can be colonised for only a limited period, the spatial pattern continually shifts with time. As a result, the beetles are faced in each generation with the problems of relatively long-distance dispersal and host-finding. The problems are greater in tropical than temperate forests because of the higher species diversity and the more rapid processes of decay. In a highly heterogeneous environment, it can be advantageous to evolve strategies that reduce the apparent heterogeneity of the system from the point of view of the individual insect. One such strategy is to increase the degree of polyphagy[23].

The finding that host specificity of phloeophagous species is greater in Fiji than in West Malaysia is also contrary to current thought. Birds on islands tend to have broader habitats and eat more kinds of food than on the mainland[8,25]. For the Fijian beetles, it is useful to distinguish between endemic and introduced species. The former are more host specific than the latter, and tend to be confined to the native forest trees[18]. The introduced species are mostly 'tramp' species which have been transported over wide areas of the tropics and are able to breed in both native and introduced trees. The greater host specificity of the endemic species may be related to the lower diversity of Fijian than Malaysian forests. From the point of view of the beetles, the Fijian forest is less heterogeneous and greater specialisation is possible. The introduced species with broader niches seem likely to gradually displace the endemic specialists[26,27].

The reasons for the low proportion of xylomycetophagous species in temperate areas, and of phloeophagous species in tropical areas are not clear[20]. The ambrosia fungi presumably grow more slowly in colder climates, and the rate of production may be insufficient to keep the beetles and their offspring alive. The number of phloeophagous species in the tropics may be reduced by the greater abundance of Cerambycidae (longhorn beetles), many of which occur in the same niche, and whose larvae act as predators of bark beetle broods. Other factors are doubtless involved.

The data presented above do not support the usual view that species are more specialised in the tropics. Other animal taxa whose essential resources are more scattered in space and/or time in tropical than temperate latitudes may well show 'reverse' latitudinal gradients too. There is so little information available on the host specificity of invertebrates along latitudinal gradients that generalisations are premature.

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Swimming control in a cubomedusan jellyfish

R. A. Satterlie & A. N. Spencer

Department of Zoology, The University of Alberta, Edmonton, Alberta, Canada T6G 2E9

Although it has been established that swimming in scyphozoans is under control of rhopalial pacemakers and an associated subumbrellar nerve-net[1–3], little is known about this control at the cellular level. There is some doubt whether scyphozoan neurones have all-or-none action potentials[3–6]. Here we show that subumbrellar neurones of the sea wasp *Carybdea rastonii* Haacke (Class Scyphozoa, Order Cubomedusae) propagate such action potentials, and that activity in these neurones originates in the rhopalial pacemakers and produces a contraction of the swimming musculature. The neurophysiology of swimming has not previously been studied in this order.

In order to determine the structure of the subumbrellar nerve-net of *Carybdea* we examined both live material and electron micrographs. Bipolar and tripolar neurones form an apparently random network throughout the subumbrella of *Carybdea*. The diameter of neurites ranges from 0.2 to 5 μm while the cell bodies can be up to 40 μm long. Interneural and neuromuscular synapses occur throughout the subumbrellar nerve-net.

Pacemaker activity is restricted to the rhopalia since jellyfish stopped swimming when we removed the rhopalia. When suction electrodes were attached to a rhopalium and to an adjacent region of the subumbrella, motor impulses originating in the rhopalium and conducted into the subumbrellar nerve-net were recorded. Potentials recorded over the nerve-ring during swimming consisted of two distinct components; a rapid (<10 ms) invariable biphasic spike, up to 80 μV, and a complex multiphasic spike of variable amplitude and duration (Fig. 1*a*). Changes in amplitude of the second component were associated with variations in the magnitude of muscular contractions (Fig. 1*b*). Therefore, we interpret the two components as an initial nerve spike followed by a compound muscle potential. As the interpulse interval of pacemaker spikes decreases, the amplitude of muscle potentials and contractions increases, suggesting neuroeffector facilitation. Excess Mg^{2+} (1:1 solution of

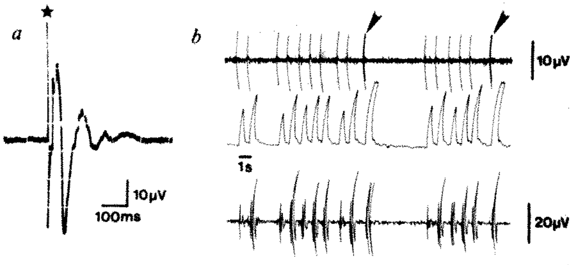

Fig. 1 *a*, An extracellular suction electrode covering the nerve-ring and surrounding subumbrellar muscle records a single, spontaneous swimming contraction. The rapid biphasic nerve spike (★) is followed by a complex muscle potential. *b*, Extracellular recordings from a rhopalial pacemaker site (top trace) and the nerve-ring of the opposite bell quadrant (bottom trace). An uncalibrated force transducer record shows circular muscle contractions at a point midway between the two electrodes (middle trace). The amplitude of the muscle contractions can be roughly correlated with the size of the compound muscle potentials of the bottom trace. Two double pacemaker spikes (arrows) give rise to double muscle potentials and a far larger contraction.

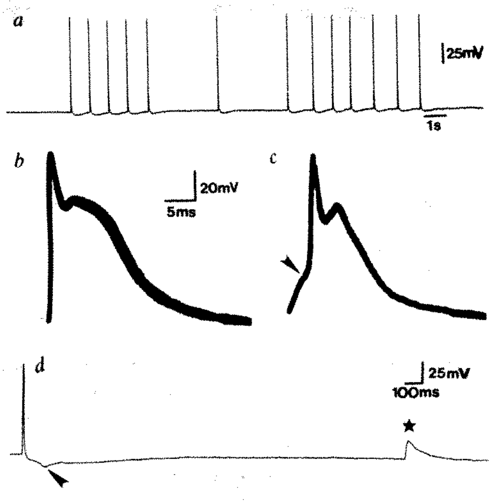

Fig. 2 *a*, Spontaneous intracellular action potentials from a subumbrellar motoneurone soma initiating 13 swimming contractions. Note the flat resting potential and the hyperpolarisations which follow each spike, *b*, Six superimposed action potentials from a motoneurone soma. In any one recording, there is little variation in the shape of the spike. *c*, The rapid depolarisation of a neuronal action potential was frequently superimposed on an apparent synaptic event (arrow). Similar synaptic events were occasionally recorded alone (*d*, ★). The notch seen in the spike after-hyperpolarisation (*d*, arrow) is a movement artefact caused by contraction of the surrounding muscles. d.c. amplification was used with 20–60 MΩ, 3 M KCl filled micropipette electrodes. The subumbrellar muscle sheet was dissected from one quadrant of the animal and pinned to a Sylgard base. A small circle of pins surrounded the penetrated neurone soma, which was visible with oblique substage illumination.

0.37 M $MgCl_2$: seawater) in the bath blocked muscular contractions within 5 min. This was followed by total cessation of all recordable electrical activity.

Cell bodies of bipolar and tripolar subumbrellar neurones gave stable recording for up to 15 min, when impaled with glass microelectrodes filled with 3 M KCl. Negative resting potentials of 72–76 mV were interrupted by all-or-none, positive-going action potentials of 105–120 mV and 12–20 ms duration (Fig. 2*a*, *b*). The slow membrane potential oscillations that have been recorded from the 'giant' neurones of hydromedusae[7,8] were not seen. Action potentials consisted of a rapid rise to peak amplitude (1–2 ms risetime) and a slow partial decay with a second rounded peak (Fig. 2*b*, *c*). The action potential was followed by a 700–800 ms, 3–15 mV after-hyperpolarisation (Fig. 2*a*). In all but one record, in which a poor penetration was suspected, little variation in the shape of the action potential was evident during repetitive activity (Fig. 2*b*). In particular, the second peak did not vary with the strength of muscular contractions. The initial rise of many spikes was superimposed on a small 10–30 mV pre-potential (Fig. 2*c*). Occasionally similar depolarisations (200–300 ms duration) were recorded which did not trigger action potentials (Fig. 2*d*). We presume that these small amplitude events represent excitatory synaptic input from adjoining neurones of the net. Each action potential preceded a muscular contraction of the surrounding subumbrellar sheet, as judged by visual observation. Only occasionally were contractions not accompanied by spikes in the impaled neurone. In these instances, a conduction block was suspected, owing to the extensive pinning required to minimise movement near the electrode.

Those properties of the neuromuscular system of *Carybdea* which most clearly show it to be of the scyphozoan type are as follows. First, if the rhopalia, containing the pacemakers, are removed then there is no activity in the subumbrellar nerve-net and swimming ceases. Second, the flat resting potential (absence of pacemaker potentials) of subumbrellar neurones, together with the observation that action potentials in these cells seem to be driven by excitatory synaptic potentials, suggest that the subumbrellar neurones are not autorhythmic but merely pass the motor pattern on to neighbouring cells of the net. These neurones function as both interneurones and motorneurones since spiking in subumbrellar neurones is associated with contractions of the underlying musculature.

Intracellular and extracellular electrophysiological recordings indicate that information in the subumbrellar nerve-net of *Carybdea*, and possibly other scyphozoans[4,5], is frequency coded with all-or-none impulses. The neuromuscular characteristics of swimming in *Carybdea*, described here, indicate that the Cubomedusae are closely related to other scyphozoans. This conclusion may be significant since the systematic position of cubomedusae has recently been questioned[9].

All-or-none action potentials were recorded recently by Schwab in the subumbrellar neurones of another scyphozoan, *Cyanea* (personal communication).

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Fast and slow myosin within single skeletal muscle fibres of adult rabbits

H. Lutz, H. Weber, R. Billeter & E. Jenny

Institut für Pharmakologie und Biochemie der Universität Zürich,
Winterhurerstr. 260, CH-8057 Zürich, Switzerland

There is good evidence for the coexistence of different myosin types both in developing muscles[1–3] and in Purkinje cells from adult chicken hearts[4]. In skeletal muscle fibres of adult animals, however, coexistence of fast (FM) and slow (SM) myosin has only been demonstrated after long-term electrical stimulation[5,6]. The term 'promiscuity' has recently been coined[7] to describe the coexistence of different myosin isoenzymes within a single fibre. Using novel, refined immunological methods we demonstrate here the presence of both FM and SM within single fibres of the musculus tibialis anterior of adult rabbits. Essentially identical results were also obtained with other muscles. Our findings imply that the genes coding for FM and SM can be expressed simultaneously within the same cell throughout an animal's entire life, and not only during development or after artificial electrical stimulation.

To test for the presence of FM and SM, antibodies of high specificity and high affinity[8] were raised against rabbit FM, SM and the light chains of rabbit cardiac myosin[9,10]. These antibodies were used in two types of assay. First, immunohistochemistry for myosin was performed on sections of shock-frozen muscle pieces and the reaction monitored by histochemical photometry. In addition, conventional histochemistry for myosin-ATPase[11], NADH-tetrazolium reductase[12] and phosphorylase[13] allowed the fibres to be classified into fibre types[11,14]. Combining the nomenclatures proposed by Brooke and Kaiser[11] and Peter *et al.*[14], the fibres were designated as type I (slow-twitch-oxidative), type IIA (fast-twitch-oxidative-glycolytic), type IIB (fast-twitch-glycolytic) and type IIC (the very

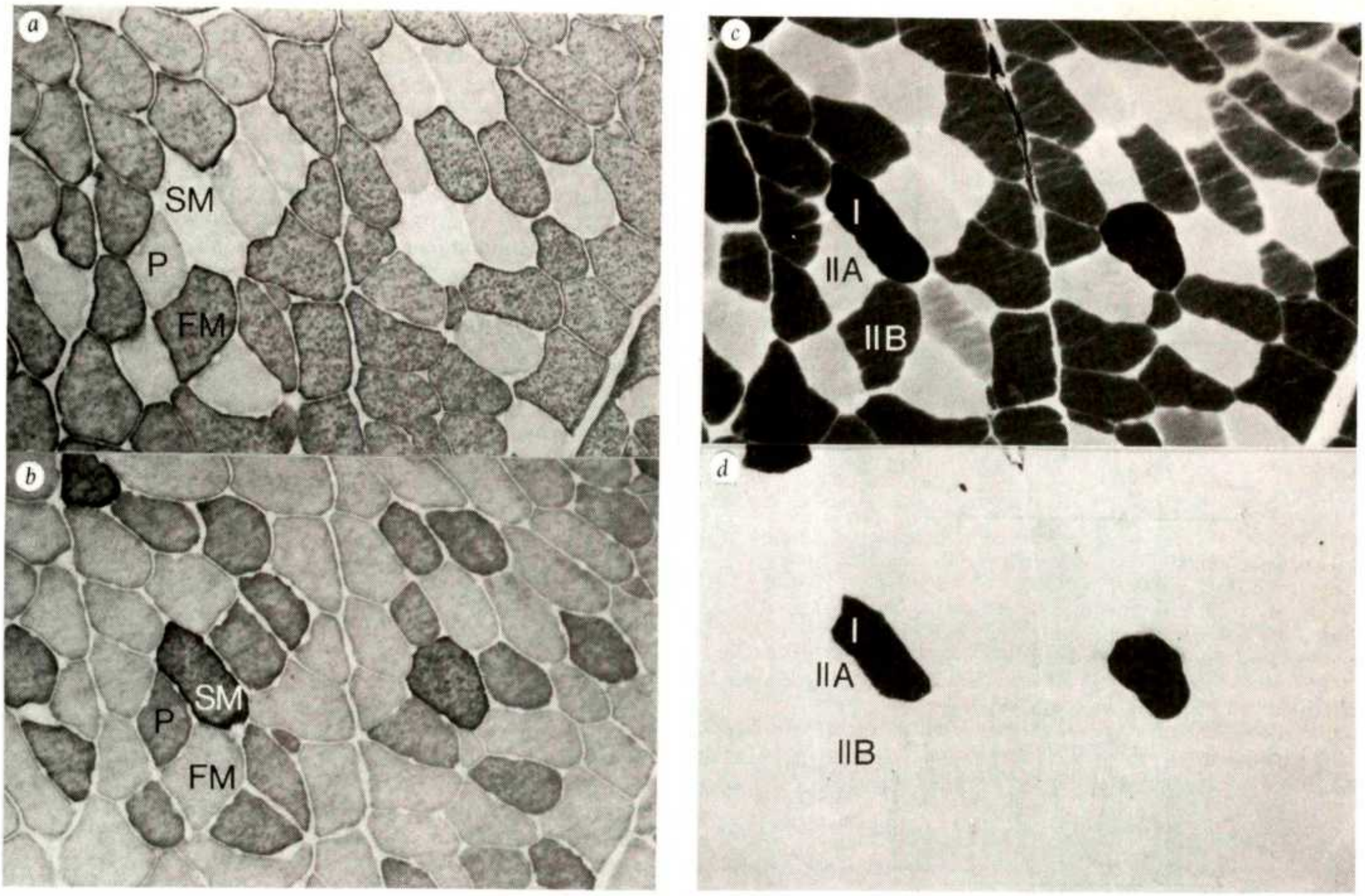

Fig. 1 Successive serial transverse sections of the musculus tibialis anterior of an adult (18 months old) rabbit analysed for FM (*a*), SM (*b*) and histochemical fibre type (*c*, *d*). Antibodies of high affinity and low heterogeneity were induced in high-responder guinea pigs using a long-term, low-dose immunisation programme[8] and their specificities demonstrated as described[9]. Antibodies against the light chains of rabbit cardiac myosin were also prepared[10] and analysed in the same way. Cryostat sections (8–10 μm) of shock-frozen muscle pieces were reacted with antibody and the immuno-complexes visualised using a protein A-peroxidase technique optimised for our needs[18]. *a*, Reaction with anti-FM serum. *b*, Reaction with anti-SM serum; the same staining pattern was obtained with anti-cardiac-light-chain sera shown to cross-react strongly with the two light chains of SM but only negligibly with those of FM (Fig. 3*a*, *b*). *c*, *d* Staining for myosin-ATPase[11] after preincubation at *p*H 4.6 or 4.35, respectively, for 5 min as a means of classifying fibres into types I, IIA, IIB and IIC; identical results were obtained when staining was for NADH-tetrazolium reductase[12] and phosphorylase[13] (data not shown). Note that some fibres, one of them marked P (promiscuous), react with both antisera (*a*, *b*); histochemically they belong to type IIA (*c*). ×150.

rare type IIC fibres have been designated as undifferentiated fibres[15]). Second, myosin light chains from dissociated muscle tissue were analysed by combining classical gel electrophoresis[16] with subsequent assay of the separated proteins by an enzyme-linked-immunosorbent-assay (ELISA) technique[9].

When serial sections of the anterior tibial muscle were reacted with sera raised against FM (Fig. 1*a*) or SM (Fig. 1*b*) and the fibres histochemically typed (Fig. 1*c*, *d*), a clear correspondence of the staining patterns emerged. Type I fibres reacted with anti-SM sera only and type IIB fibres with anti-FM sera only. In contrast, type IIA fibres reacted with both antisera, as did the rarely occurring type IIC fibres. Close examination of the staining intensities of type IIA (P) fibres (Fig. 1, *a*–*c*) indicated the existence of subpopulations containing varying proportions of FM and SM. Computer-assisted analysis of the staining intensities of 132 randomly chosen fibres reacted with anti-FM and anti-SM sera revealed three well separated clusters (Fig. 2) corresponding to fibres containing only SM (7.3% of all fibres), only FM (61.4%) or both SM and FM in varying proportions (31.3%). Our results agree with those of Spamer and Pette[17], who presented evidence suggesting the existence of subpopulations of type II fibres having a wide range of aerobic oxidative capacities and differences in their ability to oxidise fatty acids.

To confirm the presence of SM within type II fibres, 10 serial 40-μm sections of the anterior tibial muscle were selected for absence of type I fibres. Following dissociation, the released proteins were separated by gel electrophoresis and analysed

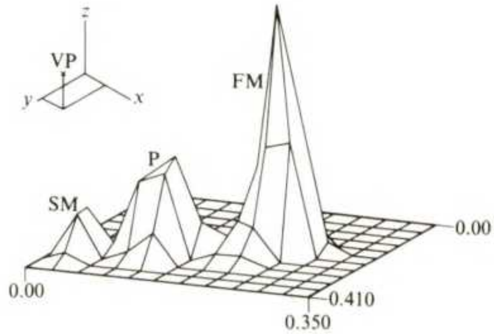

Fig. 2 Computer-assisted analysis of the FM and SM contents of 132 randomly chosen fibres from the anterior tibial muscle of an adult (18 months old) rabbit. Two successive transverse cryostat sections (8–10 μm) were reacted with anti-FM or anti-SM, respectively, and developed using a protein A-peroxidase technique[18]. Protein A binds to the Fc portion of immunoglobulins (IgG) and the covalently attached peroxidase thus allows detection of antigenic sites. The absorbance of each fibre was measured (Leitz microscope photometer MPV 2), computed and plotted to give a relative frequency distribution. *x*, Absorbance after reaction with anti-FM serum. *y*, Absorbance after reaction with anti-SM serum. *z*, Relative frequency of absorbance values. VP, computer adjusted viewpoint for optimal three-dimensional impression. P, fibres containing both FM and SM; the width of this peak suggests the presence of subpopulations of fibres containing varying proportions of FM and SM.

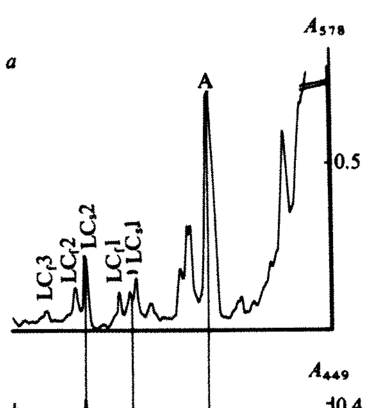

Fig. 3 Analysis of myosin light chains by a combination of 12% polyacrylamide gel electrophoresis and ELISA. *a*, Densitogram of gel stained with Coomassie brilliant blue after electrophoresis[16] of an artificial test mixture obtained from dissociated (5 min at 98 °C in SDS sample buffer) myofibrils of fast and slow twitch fibres (~20 μg of protein each). *b*, Immunoreaction of the same proteins as in *a*: proteins were eluted from slices (1 mm segments) of a parallel gel, reacted with antiserum (dilution 1:100) raised against the light chains of rabbit cardiac myosin and assayed by an ELISA technique[9]; this antiserum reacted negligibly with light chains from FM alone but gave a trace identical to the one shown here with light chains from SM alone (not shown); anti-SM sera were not used in this assay as they only recognise LC$_s$1 (ref. 9). *c*, Densitoram of gel stained with Coomassie brilliant blue after electrophoresis of total proteins from selected segments of anterior tibial muscle (adult rabbit, 18 months old); shock-frozen muscle pieces were serially sectioned (40 μm), the fibres typed histochemcally in every fifth section, and 10 intervening sections chosen for absence of type I fibres; the sections were dissociated as in *a* and the proteins analysed by electrophoresis. *d*, Immunoreaction of the same proteins as in *c* separated on a parallel gel and analysed by an ELISA technique[9] (serum dilution 1:75) as in *b*. Densitograms in *a* and *c* were aligned with respect to actin. A, actin; LC$_f$1, LC$_f$2 and LC$_f$3, light chains of FM; LC$_s$1 and LC$_s$2, light chains of SM.

using an antiserum directed against the light chains of SM (Fig. 3*a*, *b*). This revealed two main peaks corresponding exactly to the positions of the two light chains of SM (Fig. 3*c*, *d*). This result supports the view that type II fibres also contain SM. However, identification of the SM-containing subtype(s), for example types IIA, IIB or IIC, was not possible.

There is already evidence that fibres contain both FM and SM. In rat diaphragm, Gauthier *et al.*[1] occasionally detected fibres reacting with both anti-FM and anti-SM sera. In the anterior tibial muscle of rabbits we[9] and Gröschel-Stewart[19] found three populations of fibres, one of them giving an intermediate reaction with antibodies raised against either human pectoral muscle actomyosin or rabbit FM, respectively. In addition, reciprocal transformations of fibre types occurred in rats[20] and humans[21] depending on physical activity, suggesting a profound change in the proportions of myosin isoenzymes. However, in histochemically identified single fibres of adult rabbit skeletal muscles, Weeds *et al.*[22] and Pette and Schnez[23] could only demonstrate the presence of either FM or SM by microgel electrophoresis. Rubinstein *et al.*[6] did not find fibres staining with anti-FM and anti-SM sera by direct immunofluorescence. These seemingly contradictory results can be explained if we consider the sensitivities of the methods used. We believe that a suitable sensitive immunological method can detect a smaller amount of one myosin species in a mixture of two than microgel electrophoresis of isolated single muscle fibres[22,23]. The direct immunofluorescence technique[6] has its well known limitations; we found that the more sensitive indirect immunofluorescence method was insufficient to detect unambiguously the presence of fibres containing both FM and SM[9]. From this we conclude that, in general, type IIA fibres probably contain very small amounts of SM.

An alternative explanation of our results could be the occurrence, in fibres reacting with both FM and SM antisera, of a hybrid myosin consisting of FM heavy chains and SM light chains. Such an hypothetical hybrid in adult muscles has been proposed by Hoh[24]. Hybrids in developing muscles have been suggested by Rubinstein *et al.*[2] and by Pette *et al.*[25].

The results reported here demonstrate that the presence of FM and SM within single muscle fibres of adult animals occurs naturally. Weeds[7] has recently pointed out that isoenzymes of myosin should provide useful markers for unravelling the early events in myogenesis and the differentiation of muscle fibres following innervation. In view of our recent observation that the myosin composition of muscle fibres changes with physical training (R.B., unpublished observation) and during ageing[26], we may add that the analysis of myosin isoenzyme composition could help in understanding the plasticity of muscle throughout life.

Since submission of this manuscript the presence of FM and SM within single fibres of adult rat soleus muscle has been demonstrated[27].

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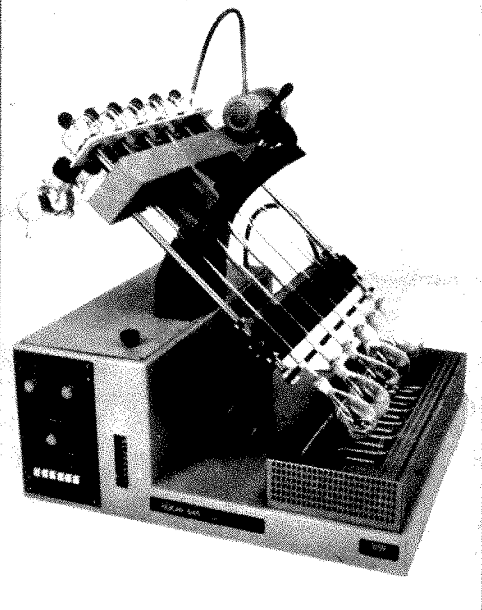

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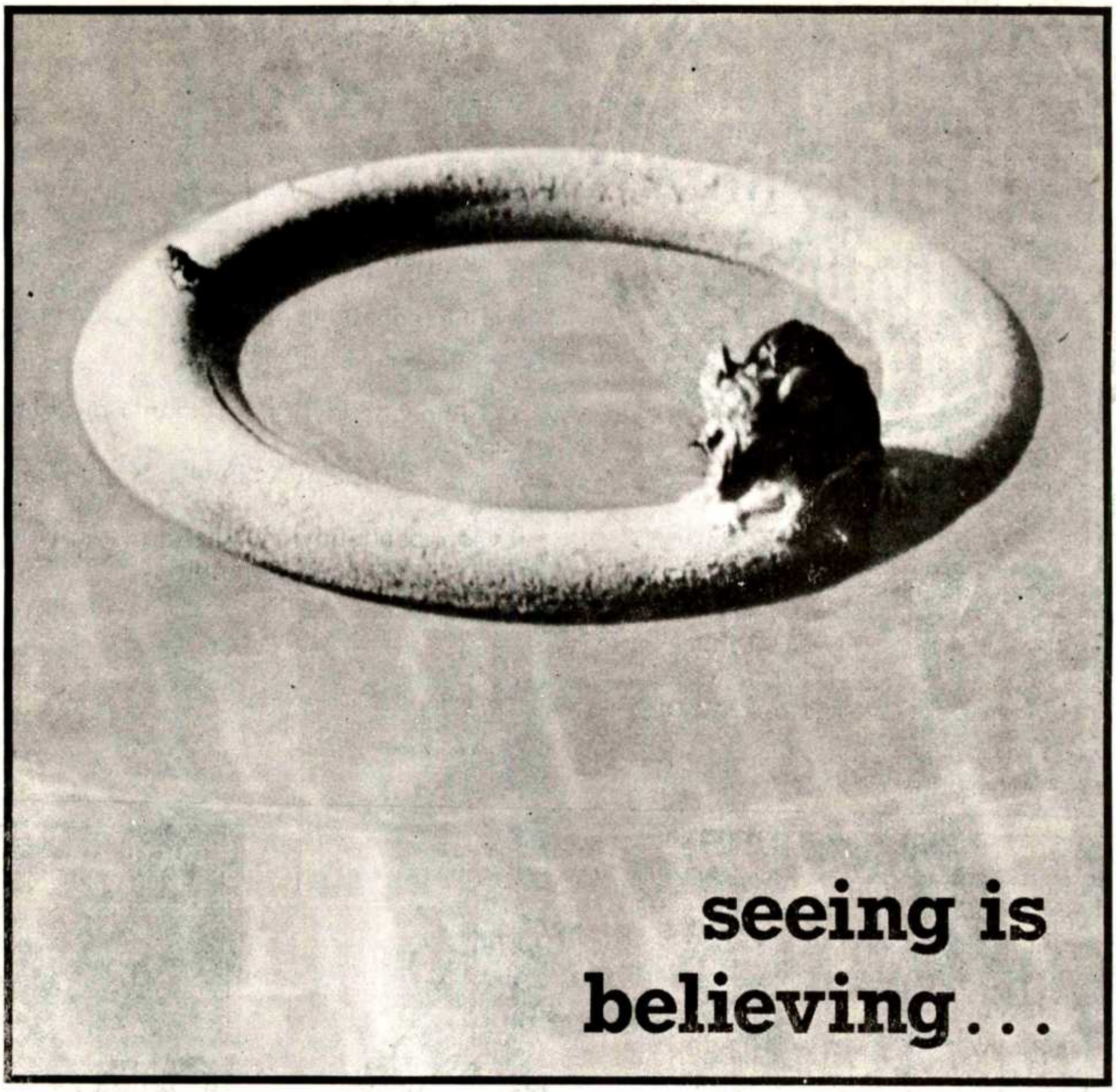

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'Square arrays' in the sarcolemma of human skeletal muscle fibres

H. Schmalbruch

Institute of Neurophysiology, University of Copenhagen,
Copenhagen, Denmark

Fast twitch fibres of rat[1] and rabbit[2] show rectangular patterns of intramembrane particles in freeze-fracture preparations of the sarcolemma. These 'square arrays' are almost totally absent in the slow twitch soleus muscle of rat[1]. I report here differences in the incidence of square arrays in human fetal and adult muscle and in different fibres within a human muscle. Square arrays probably classify fast and slow twitch fibres in freeze-fracture preparations of mixed muscles.

A fetal brachial biceps muscle was obtained from a male fetus weighing 385 g (20–24th week of gestation). Surgical biopsies were taken from the medial vastus muscle of two normal male subjects 29 and 36 yr old; the site of biopsy was close to the motor point. Specimens fixed in 2.5% glutaraldehyde were fractured at −100 °C in a Balzers BAE 120 unit and replicated without etching.

Square arrays were found in all fibres from adult muscles but in none of the fetal muscle fibres. The arrays consisted of particles, 6–7 nm in diameter, attached to the P-face of the plasma membrane (Fig. 1); the E-face showed corresponding pits. In some fibres square arrays occurred in clusters and the individual arrays might contain up to 60 particles, whereas in other fibres arrays were dispersed randomly and rarely contained more than 20 particles. The numbers of square arrays per μm² plasma membrane determined in individual fibres formed a two-peak histogram; in fibres in which square arrays occurred frequently the individual arrays were larger than in fibres in which square arrays were rare (Fig. 2).

Thus, the frequency and size of square arrays of the plasma membrane of adult muscle fibres in man identify two classes of fibres; both are different from fetal muscle fibres. This was not due to the fact that junctional and non-junctional parts of the membrane were compared, because none of the preparations contained endplates. From the findings in fast and slow twitch muscles in rat[1] I conclude that the fibres with large square arrays that constitute the right peak of the histogram (Fig. 2) are fast twitch fibres and that those with smaller arrays that constitute the left peak are slow twitch fibres. This difference is less obvious than in rat muscles, where fast twitch fibres display 5–15 square arrays per μm² and slow twitch fibres almost none[1]. The fraction of fibres that were presumably fast (29 of 51, Table 1) agrees with the percentage of fibres that in frozen sections stained intensely for ATPase at *p*H 9.4 (52% and 57%, respectively). In normal mature muscle an intense reaction is thought to identify fast twitch fibres. In the fetal muscle studied all fibres reacted uniformly with quite high intensity.

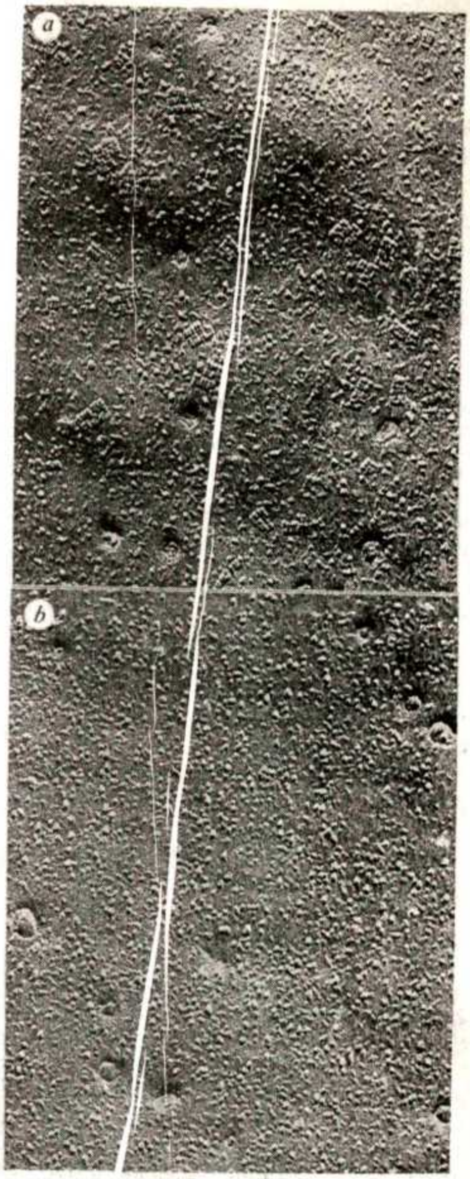

Fig. 1 Freeze-fractures of the medial vastus muscle of man. Both micrographs were taken from the same preparation, the direction of shadowing is from below. The P-faces of the sarcolemma of presumed fast twitch (*a*) and slow twitch (*b*) fibres are shown. Note the many orthogonal arrays of particles in *a* which are absent in *b*. Of each fibre, 22 μm² of the plasma membrane was studied; the number of square arrays was 11.1 per μm² and 1.3 per μm² in the fibres shown in *a* and *b*, respectively. Particles not contained in square arrays vary in diameter. The shallow invaginations represent openings of T-tubules or of caveolae of the plasma membrane. (×72,000).

Table 1 Quantitative findings in the P-face of the sarcolemma of human muscle fibres

	Adult muscle		Fetal muscle	Final magnification of micrographs
	Presumed fast twitch fibres	Presumed slow twitch fibres		
Square arrays per μm² mean (s.d.; *n* fibres)	5.9(3.07; 29)	1.3(0.85; 22)	—	×60,000
Intramembrane particles not in square arrays				
Particles per μm²	949	909	929	×400,000
Size (nm) mean (s,d,; *n* particles	9.7(1.9; 247)	9.4(1.3; 237)	10.5(2.4; 223)	×400,000
Caveolae per μm² mean (s.d.; *n* micrographs)	11.6(4.4; 40)	15.5(7.2; 32)	7.1(3.3; 10)	×60,000

In adult muscles, fibres were classified as presumably fast or slow twitch by size and arrangement of square arrays (unhatched and hatched areas in Fig. 2).

Intramembrane particles attached to the cytoplasmic fracture face (P-face) but not contained in square arrays had the same diameter and were similar in number in fetal and adult and in fast and slow twitch fibres (Table 1). The differences in size were at most 1 nm and thus well below the resolution and reproducibility of the method. The number of this sort of intramembrane particles was higher (900 per μm²) than in previous studies (250 per μm²)[3–5]. In these studies, particles were counted at low magnification (×60,000)[4,5] and small particles may have been

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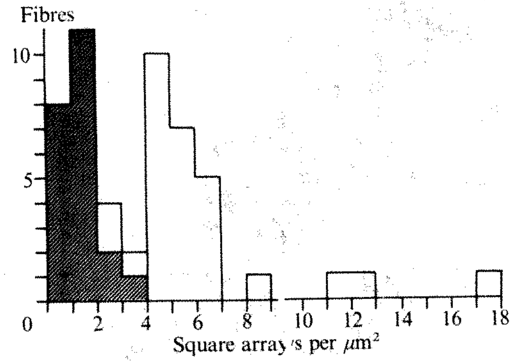

Fig. 2 Histogram of the frequencies of square arrays in 51 randomly selected fibres. Of each fibre, 22 μm^2 of the P-face of the sarcolemma was investigated. The hatched area represents fibres with small and scattered square arrays, the unhatched area represents fibres with large and clustered square arrays.

overlooked. The size was not measured. Although it is possible that some intramembrane particles are artefacts due to condensation of water before shadowing, it is unlikely that the discrepancy is due to the fact that condensation of water was more pronounced in this than in the previous studies.

In rat soleus muscles cross-innervated by a nerve to a fast muscle, square arrays become more frequent; denervation alone does not change the number of square arrays[6]. As cross-innervation changes the contractile properties and the type of myosin[7], this might suggest that innervation exerts a coordinated control of membrane and cytoplasmic aspects of muscle differentiation. Nevertheless, it is unlikely that the type of myosin and number of square arrays are determined by the same factor because fetal muscle fibres that lack square arrays contain a type of myosin that corresponds to myosin in fast twitch fibres of adult muscle[8-12].

There is evidence that intramembrane particles are proteins that, at least partly, determine the physiological properties of the plasma membrane; these properties differ in mature and immature and in fast and slow twitch fibres[13-19]. The number and size of particles not contained in square arrays were the same in the three sorts of fibres studied. This indicates either that square arrays, that is, the unknown protein constituting the particles within these arrays, determine specific properties of the membrane, or that structural differences of particles not contained in square arrays were too small to be demonstrated.

To speculate on the function of square arrays requires a comparison of findings in different species. Structures resembling square arrays are abundant in astrocyte membranes rich in ($Na^+ + K^+$)ATPase; hence, it has been suggested that the number of arrays is related to the activity of this enzyme in muscle fibres[1]. The activity of ($Na^+ + K^+$)ATPase, however, is the same in plasma membranes of fast and slow twitch fibres[20]. One could relate square arrays to a particular type of sodium channel, because only in mature, not immature, muscle fibres are action potentials blocked by tetrodotoxin[18]. Finally, one might consider the distribution of acetylcholine receptors, which is related strictly inversely to that of square arrays: in immature fibres, receptors are randomly distributed and square arrays are absent[15], in fast twitch fibres, receptors are concentrated in the endplate region[21] and square arrays occur everywhere except in the endplate region[1], and in slow twitch fibre some receptors also occur in the extra-junctional membrane[15] where square arrays are less frequent than in fast twitch fibres.

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Use of chlorotetracycline fluorescence to demonstrate Ca^{2+}-induced release of Ca^{2+} from the sarcoplasmic reticulum of skinned cardiac cells

Alexandre Fabiato & Françoise Fabiato

Department of Physiology, Medical College of Virginia, Richmond, Virginia 23298

It has been proposed that the trans-sarcolemmal influx of Ca^{2+} occurring during the plateau of the mammalian cardiac action potential is insufficient in itself to activate the myofilaments, but can trigger a release of Ca^{2+} from the sarcoplasmic reticulum (SR) which is sufficient for activation[1]. The demonstration of this Ca^{2+}-induced release of Ca^{2+} relied entirely on experiments in which the tension developed by the myofilaments was used as a sensor of the changes of myoplasmic free Ca^{2+} concentration ([free Ca^{2+}]) in segments of single cardiac cells from which the sarcolemma had been removed by microdissection (skinned cardiac cells)[1]. The small size of these preparations has previously prevented the use of more direct methods for the detection of myoplasmic Ca^{2+} movements[2-4]. The present study is a direct demonstration of Ca^{2+}-induced release of Ca^{2+} from the SR of skinned cardiac cells treated with chlorotetracycline (CTC), a fluorescent chelate probe which enables changes in the amount of Ca^{2+} bound to a variety of biological membranes or micelles to be monitored[5-8]. The fluorescence increases when more Ca^{2+} is bound.

Skinned cardiac cells, 10 μm wide and 50 μm long, from the rat ventricle were prepared as described previously[1]. It was essential to limit the duration and intensity of illumination used for fluorescence excitation so as to avoid a progressive deterioration of the Ca^{2+} accumulation in and release from the SR. Microdissection and attachment of the skinned cell to the microtools were carried out using the regular low-voltage transmitted light of the Biovert inverted microscope (Reichert). Then, in the absence of any illumination, the skinned cell was superfused for 10 min with 5.0×10^{-5} M CTC in a solution containing 3.2×10^{-8} M free Ca^{2+} and 3.2×10^{-4} M free Mg^{2+}, at pH 7.10 and 22 °C. To eliminate any contractile movement during Ca^{2+} release from the SR which would have disturbed the optical recording, a high concentration (1.0×10^{-3} M) of EGTA was used in all the solutions. The [free Ca^{2+}] of 3.2×10^{-8} M has been shown to result in only slightly less than optimal Ca^{2+} loading of the SR[9]. The cell was then washed for 5.0 s in the same Ca^{2+}-containing solution but in the absence of CTC. Only at this time was the shutter open; the fluorescence was excited with the incident illumination of the Biovert microscope through an interference filter (with peak transmission at 400 nm and a bandwidth of 4 nm at half height), a heat absorption filter and an exciter filter. The fluorescence emission was collected with a

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×60 objective (0.90 n.a.) through a monochromator adjusted at 530 nm, and was measured with a photomultiplier as part of a modified high-sensitivity Reichert microspectrophotometer.

When the perfusion containing 3.2×10^{-8} M free Ca^{2+} was replaced by one with 1.8×10^{-7} M free Ca^{2+}, there was a rapid decrease in fluorescence (Fig. 1*a*). Perfusion with 1.0×10^{-8} M free Ca^{2+} caused a slight increase in fluorescence, and 3.2×10^{-8} M free Ca^{2+} a larger fluorescence. In contrast, a further increase in [free Ca^{2+}] to 1.8×10^{-7} M caused an abrupt decrease in fluorescence.

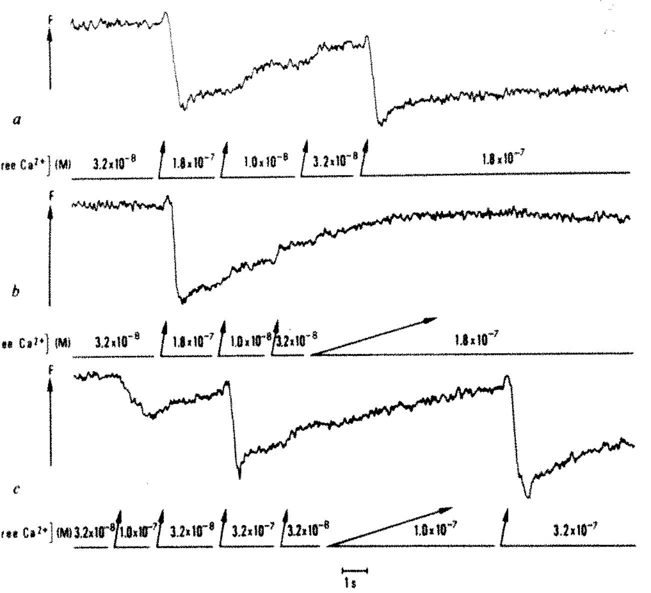

Fig. 1 Effects of varying the myoplasmic [free Ca^{2+}] in skinned cardiac cells on the fluorescence of chlorotetracycline (US Biochemical Corporation). The arrow labelled F at the left of each tracing indicates an increase in fluorescence and the size of the arrow corresponds to 5% of the fluorescence measured during the initial perfusion with 3.2×10^{-8} M free Ca^{2+} (measurement done during a 1.0-s opening of the shutter, not shown). The arrows below each tracing indicate the time and rate of the solution changes. A slow variation of the [free Ca^{2+}] between two values was obtained by varying the relative output of two simultaneous perfusions with two solutions of [free Ca^{2+}] equal to the initial and terminal values, respectively. The perfusion solutions contained 1.0×10^{-3} M total EGTA, 3.2×10^{-3} M Mg-ATP, 3.2×10^{-4} M free Mg^{2+}, 1.5×10^{-2} M creatine phosphate, 15 U ml^{-1} creatine phosphokinase, 0.160 M ionic strength with K^+ and Cl^- as major ions, and 1.0×10^{-1} M sucrose. The *p*H was maintained at 7.10 with 3.0×10^{-2} M *N*-tris(hydroxylmethyl)methyl-2-aminoethanesulphonic acid (TES), which is sufficient for *p*H buffering during Ca^{2+} release from the SR of skinned cardiac cells[9]. The stability constants and the computer program used in the calculation of the multiple equilibria between metals and ligands have been reported in detail elsewhere[9]. In particular, the stability constant described by Allen and Blinks was used for the Ca–EGTA complex (see ref. 9 for refs and correspondence to other stability constants). An apparent stability constant of 7.53×10^2 M^{-1} from the Ca–CTC complex was included in the computer program although this binding is much smaller than the binding of EGTA to Ca. Experiments similar to that of *a* except that the 5.0 s washing and the increase in [free Ca^{2+}] from 3.2×10^{-8} M to 1.8×10^{-7} M were applied at various intervals after the beginning of the CTC-containing perfusion (and experiments using caffeine for inducing a fluorescence decrease) demonstrated that the mean value of the half-time of the CTC perfusion necessary to obtain a maximum amplitude of transient decrease of fluorescence was 176 s. During a continuous perfusion with 3.2×10^{-8} M free Ca^{2+} in the absence of the CTC, there was a progressive decrease in the amplitude of the transient fluorescence decrease. The half-time for this decrease was 142 s when the preparation was illuminated by opening the shutter only at the time of the increase of the [free Ca^{2+}] to 1.8×10^{-7} M. The consistency between the half-times for the increase (176 s) and decrease (142 s) in the amplitude of the fluorescence transient suggests that the decrease was mainly caused by a progressive leakage of CTC from the SR. Additional mechanisms such as a weak Ca^{2+}-ionophoretic effect of CTC[17] could have a minor role. However, during continuous illumination the amplitude of the fluorescence transient decreased much faster because of photodynamic damage to the Ca^{2+} accumlation in and release from the SR. Accordingly, conclusions were derived only from experiments in which, as in *c*, the amplitude of the transient was larger during the second triggering than during the first one. The findings would have been even more dramatic if corrections for the progressive deterioration had been made. Finally, the simplest explanation for the small and transient increase in fluorescence that preceded the Ca^{2+}-induced decrease is to attribute it to the effect of reaching myoplasmic [free Ca^{2+}] values itermediary between 3.2×10^{-8} M and 1.8×10^{-7} M during the time required for perfusion change (0.2 s). This would explain the finding that the fluorescence increased further until a [free Ca^{2+}] sufficient to trigger Ca^{2+} release had been reached.

Although CTC binds to Mg^{2+} as well as to Ca^{2+}, the wavelength of 400 nm selected for excitation favoured the fluorescence of the Ca^{2+} chelate over that of the Mg^{2+} chelate. When excitation was at wavelength 375 nm, which favours the Mg^{2+} chelate, the same increase in [free Ca^{2+}] from 3.2×10^{-8} M to 1.8×10^{-7} M produced a decrease in fluorescence of less than 1% of the maximum fluorescence (defined in Fig. 1 legend). In contrast, a decrease of [free Mg^{2+}] from 3.2×10^{-4} M to 3.2×10^{-5} M, in the presence of a constant [free Ca^{2+}] at 3.2×10^{-8} M, produced a fluorescence decrease of 3% and 14% at excitation wavelengths of 400 and 375 nm respectively. Thus, the changes of fluorescence shown in Fig. 1*a* directly monitored changes in the amount of bound Ca^{2+}, rather than changes in the amount of bound Mg^{2+} resulting from the Ca^{2+} translocation.

The changes in fluorescence intensity (Fig. 1*a*) did not correspond to changes in the amount of Ca^{2+} bound to the mitochondria because results similar to those shown in Fig. 1*a* were obtained in skinned cardiac cells treated with 5.0×10^{-3} M azide or 5.0×10^{-6} M carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone, which should inhibit the energy-linked Ca^{2+} transport by the mitochondria[10], or in cells treated with 1.0×10^{-6} M ruthenium red, which should inhibit passive Ca^{2+} binding to the mitochondria[10]. These findings are consistent with previous results from tension recordings in skinned cardiac cells suggesting that the mitochondria do not interfere with either the active transport or the passive binding of Ca^{2+} when the myoplasmic [free Ca^{2+}] is lower than 1.0×10^{-6} M (refs 1, 9). Furthermore, the classical data supporting the evidence that isolated mitochondria present high-affinity binding sites for Ca^{2+} effective at concentrations lower than 1.0×10^{-6} M have recently been claimed to be artefactual[11,12]. A rapid decrease in fluorescence similar to that shown in Fig. 1*a* could be induced by 1.0×10^{-2} M caffeine or 1.0×10^{-5} M of the ionophore A23187. Both interventions release Ca^{2+} from the SR[9]. The amplitude of the fluorescence decrease induced by increasing the [free Ca^{2+}] was about half of that induced by caffeine and one-quarter of that induced by A23187. In contrast, no decrease in fluorescence was induced by either increasing the [free Ca^{2+}] or adding caffeine or ionophore in cells treated with 0.5% of the detergent Brij 58, which destroys the ability of the SR actively to accumulate and release Ca^{2+} (refs 1, 9). Accordingly, the transient changes in fluorescence intensity (Fig. 1*a*) were entirely attributed to changes in the amount of Ca^{2+} bound by the SR. Note, however, that the preparation retained about 75% of its maximum fluorescence after treatment with Brij 58 and that about 20% of the fluorescence was background fluorescence already present before the application of CTC.

Using a Millipore filtration technique for monitoring changes in the CTC content of the medium bathing SR vesicles, Millman and Haynes[13] have recently demonstrated that CTC penetrates the SR. They concluded that both the influx and efflux of CTC are much slower than those of Ca^{2+}. These findings are consistent with the results obtained from skinned cardiac cells and summarised in Fig. 1 legend. Thus, the 10-min perfusion that preceded the tracing shown in Fig. 1*a* permitted the slow diffusion of CTC into the SR and its complexation with the Ca^{2+} bound to the SR membrane. As the [free Ca^{2+}] inside the SR attains a rapid equilibrium with the Ca^{2+} bound to the SR membrane, a decrease in the [free Ca^{2+}] inside the SR caused by Ca^{2+} release should decrease the amount of Ca^{2+} bound, and this should result in a decrease in fluorescence intensity[5–8]. Accordingly, the rapid fluorescence decreases observed in Fig. 1*a* are interpreted as a demonstration of Ca^{2+} release. Because of the slow efflux of CTC, most of the CTC that had penetrated the SR during the 10-min loading period remained trapped there after the Ca^{2+} release which caused the initial decrease of fluorescence (Fig. 1*a*). The subsequent application of solutions with increasing [free Ca^{2+}] caused a reloading of the SR with Ca^{2+} which resulted in a recovery of the fluorescence. Conversely, the sudden decrease in fluorescence observed when the [free Ca^{2+}] of the solution reached 1.8×10^{-7} M demonstrates a Ca^{2+}-induced release of Ca^{2+} from the SR.

The minimum [free Ca^{2+}] required for triggering a Ca^{2+}-induced fluorescence decrease varied between 5.6×10^{-8} M and 1.8×10^{-7} M in 22 experiments of the type shown in Fig. 1*a*. These values are within the same range as the [free Ca^{2+}] required for Ca^{2+}-induced release of Ca^{2+} from the SR previously inferred from tension recordings in skinned cardiac cells[9], and are lower than the [free Ca^{2+}] necessary to induce a detectable tension (about 1% of maximum) by the direct activation of the myofilaments, which was between 3.2×10^{-7} M and 5.6×10^{-7} M. Finally, the rate of the Ca^{2+}-induced decrease of fluorescence is in the same range as the rate of tension development in the intact rat ventricle at 22 °C.

All the experiments shown in Fig. 1 were carried out in the presence of 3.2×10^{-4} M free Mg^{2+} to permit comparison with our previous experiments on tension recordings that were done at this [free Mg^{2+}] (refs 1, 9). It has recently been suggested that the intracellular [free Mg^{2+}] of the intact muscle cell may be 10 times higher[14]. However, in eight experiments similar to that of Fig. 1*a*, but carried out in the presence of 3.2×10^{-3} M free Mg^{2+}, the [free Ca^{2+}] required to trigger a Ca^{2+}-induced decrease of fluorescence ranged between 1.0×10^{-7} M and 3.2×10^{-7} M. This was still less than the [free Ca^{2+}] required to induce a detectable tension by the direct activation of the myofilaments, which was 5.6×10^{-7}–1.0×10^{-6} M at this [free Mg^{2+}].

The preceding results (Fig. 1*a*) demonstrate directly a Ca^{2+}-induced release of Ca^{2+} from the mammalian cardiac SR. Fig. 1*b* and *c* correspond to additional findings which may help in understanding the mechanism of this process and in evaluating its relevance to the excitation–contraction coupling of the intact cardiac muscle. One of these findings is that the Ca^{2+}-induced release of Ca^{2+} depends not only on a critical level of [free Ca^{2+}] being reached in the myoplasm but also on the rate at which this change of myoplasmic [free Ca^{2+}] takes place. The initial part of Fig. 1*b* shows that increasing the [free Ca^{2+}] from 3.2×10^{-8} M to 1.8×10^{-7} M within 0.2 s caused a Ca^{2+}-induced decrease of fluorescence in this cell similar to that observed in the experiment shown in Fig. 1*a*. The cell was then subjected to the same succession of perfusions as in the experiment of Fig. 1*a* except that the second increase in [free Ca^{2+}] from 3.2×10^{-8} M to 1.8×10^{-7} M was effected very slowly, in 5.0 s. This resulted in no Ca^{2+} release but in a continued increase of fluorescence corresponding to further loading of the SR. With such a slow (5.0 s) perfusion change a minimum [free Ca^{2+}] in the range of 3.2×10^{-7}–5.6×10^{-7} M (17 experiments) was necessary to induce a Ca^{2+} release in the presence of 3.2×10^{-4} M free Mg^{2+}.

Although the mechanism of Ca^{2+}-induced release of Ca^{2+} is unknown, it was generally believed that the trigger was simply an increase of [free Ca^{2+}] outside the SR[1,2] or the gradient of [free Ca^{2+}] across the SR membrane[9]. The present results (Fig. 1*b*) indicate that this is not the case, although more experiments will be needed to define the exact relationship between the two variables—the level of [free Ca^{2+}] and the rate of change of [free Ca^{2+}]—on which the Ca^{2+} release depends. With respect to the physiological role of the Ca^{2+}-induced release of Ca^{2+} from the SR, the present findings (Fig. 1*b*) may help to explain some aspects of the force–frequency relationships of the intact heart. For example, it may be proposed that for each beat a rapid initial Ca^{2+} influx occurring during the plateau of the action potential may trigger the release of Ca^{2+} stored in the SR. This may be followed by a slower influx of Ca^{2+}, loading the SR with an amount of Ca^{2+} which will be available for release during subsequent beats.

Another finding is that the magnitude of Ca^{2+}-induced release of Ca^{2+} can be increased by at least two mechanisms: (1) an increase in the level of the [free Ca^{2+}] used as a trigger, and (2) an increase in the level of Ca^{2+} loading of the SR just before the triggering of the Ca^{2+} release. The initial part of the tracing of Fig. 1*c* shows that 3.2×10^{-7} M free Ca^{2+} induced a larger and much faster release of Ca^{2+} than was induced by 1.0×10^{-7} M free Ca^{2+}, even though the rate of perfusion change and, presumably, the level of Ca^{2+} loading were unchanged.

The end of the tracing of Fig. 1*c* shows that a slow increase in [free Ca^{2+}] from 3.2×10^{-8} M to 1.0×10^{-7} M did not induce a Ca^{2+} release (in contrast to the effect of a fast increase to the same level seen at the beginning of the tracing), but produced a continued increase in fluorescence. Note that the fluorescence remained below the level observed during the initial perfusion with 3.2×10^{-8} M free Ca^{2+} because the CTC content of the SR was the limiting factor and had decreased slowly during the experiment. However, the larger loading of the SR is unequivocally demonstrated (see discussion in Fig. 1 legend) by the greater magnitude of the fluorescence decrease induced by the rapid increase in [free Ca^{2+}] to 3.2×10^{-7} M after its slow increase to 1.0×10^{-7} M.

This gradation of the Ca^{2+} release demonstrated in Fig. 1*c* is relevant to the understanding of the mechanism of the Ca^{2+}-induced process, which must be more complex than the all-or-none process originally proposed[15]. It is also relevant to the compatibility of this process with the known gradation of cardiac contraction with the extracellular [Ca^{2+}] and with the magnitude of the trans-sarcolemmal Ca^{2+} current[16].

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Specific tricyclic antidepressant binding sites in rat brain

Rita Raisman, Michael Briley & Salomon Z. Langer

Synthélabo, LERS, Department of Biology, 58, Rue de la Glacière, 75013 Paris, France

The discovery of high-affinity binding sites for psychoactive drugs such as benzodiazepines[1,2], opiates[3] and neuroleptics[4] has opened up new approaches to the study of these drugs and their mechanisms of action. Although most tricyclic antidepressants inhibit neuronal uptake of noradrenaline and serotonin[5], their mechanism of action remains unclear. Changes in the sensitivity of the β-receptor after chronic tricyclic antidepressant treatment[6,7] suggest that they modulate noradrenergic neurotransmission. Tricyclic antidepressants also act directly on cholinergic[8], histaminergic[9], α-adrenergic[10] and serotonergic[11] receptors. It is not clear, however, which, if any, of these effects

are related to the primary antidepressant effect or whether they are simply responsible for some of the side effects. We have thus investigated the possibility that specific binding sites for tricyclic antidepressants exist in the central nervous system. So far, binding studies using ^{3}H-labelled tricyclic antidepressant drugs have only detected binding to histaminergic H_2 and cholinergic muscarinic receptors[12] and low-affinity binding[13]. We demonstrate here a population of specific high-affinity binding sites for ^{3}H-imipramine on brain membranes which may be responsible for the antidepressant effects of these drugs.

Cerebral cortex dissected from 180–250 g male Sprague–Dawley rats was homogenised in 50 volumes of buffer (50 mM Tris-HCl, *p*H 7.4, 120 mM NaCl, 5 mM KCl) and centrifuged at 30,000*g* for 10 min. The pellet was twice resuspended in the same buffer and recentrifuged. The final, washed pellet was resuspended in 33.3 volumes of buffer (final protein concentration ~3 mg ml^{-1}). The binding of ^{3}H-imipramine was measured at 0 °C after incubating 180 µl of membrane suspension with ^{3}H-imipramine (0.5–20 nM) (specific activity 29.8 Ci mmol^{-1}, NEN) in a total volume of 250 µl for 60 min. 100 µl was taken and rapidly diluted into 5 ml ice-cold buffer and immediately filtered through Whatman GF/F glassfibre filters. The filters were washed with 3 × 5 ml ice-cold buffer, dried and the radioactivity measured by liquid scintillation spectrometry. The specific binding of ^{3}H-imipramine was defined as the difference between the total binding and that remaining in the presence of 10 µM desipramine. At 5 nM ^{3}H-imipramine, specific binding was 60% of the total binding.

Specific high-affinity ^{3}H-imipramine binding was saturable (Fig. 1) whereas the nonspecific binding increased linearly with ^{3}H-imipramine concentration. A Scatchard analysis of the specific binding (Fig. 1*b*) gave a straight line, indicating a single population of non-interacting sites. The mean apparent dissociation affinity constant, K_d, was 4.0 ± 0.5 nM (mean ± s.e.m., $n = 13$) and the total number of binding sites, B_{max}, was 13.8 ± 1.3 pmol per g original wet tissue weight. Hill analysis (not shown) gave a Hill number of 1.04, indicating a lack of cooperativity. ^{3}H-imipramine binding reached equilibrium rapidly even at 0 °C (Fig. 2). The $t_{1/2}$ for association at 2.5 nM ^{3}H-imipramine was 5 min. Dissociation, induced by an excess (10 µM) of desipramine, was also rapid, complete displacement being achieved within 60 min ($t_{1/2}$, 5 min).

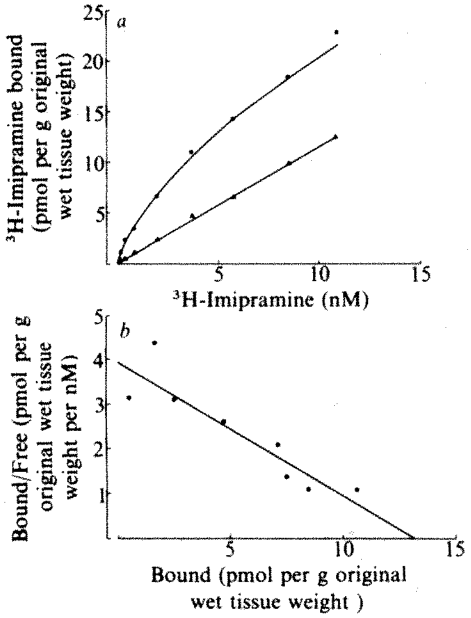

Fig. 1 Binding of ^{3}H-imipramine to rat cortical membranes. ^{3}H-imipramine at 0.1–12 nM was incubated in duplicate with or without excess desipramine (10 µM). *a*, Total binding (●) and that not displaced by 10 µM desipramine (▲) (nonspecific binding). *b*, The specific binding as a Scatchard plot ('bound', specifically bound ^{3}H-imipramine (pmol per g original wet tissue weight); 'free', free ^{3}H-imipramine (nM)).

Table 1 Inhibition of ^{3}H-imipramine binding in rat cortex

	IC_{50}(nM)		IC_{50}(nM)
Tricyclic antidepressants		Monoamine uptake blockers	
Imipramine	7	Fluoxetine	200
Desipramine	18	Nisoxetine	200
Protriptyline	20	Quipazine	700
Chloripramine	25	Cocaine	900
Amitriptyline	25		
Doxepin	300	Other drugs	
		Phentolamine	200
Atypical antidepressants		Chlorpromazine	200
		Pyrilamine	500
Iprindole	5,500	Serotonin	1,000
Viloxazine	11,500	WB 4101	2,000
Mianserin	20,000	Atropine	10,000
		Histamine	20,000

IC_{50} is the concentration required to inhibit 50% of the specific ^{3}H-imipramine binding at 5 nM. The following compounds were inactive (IC_{50} greater than 100 µM): pargyline, noradrenaline, dopamine, yohimbine, clonidine, methysergide, carbamylcholine, spiroperidol, cimetidine, Met-enkephalin, substance P, amphetamine.

The distribution of ^{3}H-imipramine binding sites varied among the major brain regions (cortex, striatum, cerebellum, hypothalamus). The greatest binding was found in the hypothalamus (K_d, 5.21 ± 3.4 nM; B_{max}, 15.97 ± 2.71 pmol per g original wet tissue weight, $n = 4$), which had seven to eight times more receptors than the region of lowest binding, the cerebellum (K_d, 8.0 ± 3.1 nM; B_{max}, 2.20 ± 0.39 pmol per g original wet tissue weight, $n = 3$). No ^{3}H-imipramine binding was detected in the heart.

All the tricyclic antidepressants studied are normally used clinically in the range of 60–300 mg per day. Five of these displaced ^{3}H-imipramine binding with very similar affinities (Table 1). Doxepin, however, was about 10-fold less potent. This difference may reflect a different absorption, distribution and metabolism for this drug *in vivo*. Tricyclic antidepressants, atypical antidepressants and monoamine uptake-blocking agents inhibited ^{3}H-imipramine binding (Table 1). However, the binding site for ^{3}H-imipramine seems to be distinct from the neuronal noradrenaline uptake site; cocaine, which blocks noradrenaline uptake, inhibits ^{3}H-imipramine binding fairly actively, but amphetamine, which *in vitro* is almost equipotent with cocaine in inhibiting noradrenaline uptake, is completely inactive at displacing the binding of ^{3}H-imipramine. Furthermore, ^{3}H-imipramime binding sites are absent in the rat heart whereas noradrenaline uptake is very active in this organ. Finally, a comparison of the inhibition of the uptake of dopamine, noradrenaline and serotonin by antidepressants with their potencies for the inhibition of ^{3}H-imipramine binding

Table 2 ^{3}H-Imipramine binding and monoamine uptake inhibition

	^{3}H-imipramine binding (IC_{50}, µM)	Uptake inhibition (IC_{50}, µM) DA	NA	5-HT
Imipramine	0.007	12.5	0.02	0.24
Desipramine	0.018	8.7	0.0015	2.24
Protriptyline	0.020	4.9	0.003	1.62
Chloripramine	0.025	3.8	0.044	0.015
Amitriptyline	0.025	5.6	0.020	—
Iprindole	5.5	7.9	2.6	10.0
Mianserin	20.0	19.0	0.084	11.0

The IC_{50} values for ^{3}H-imipramine binding are taken from Table 1. The uptake inhibition data are taken from Randrup and Braestrup[17]. DA, dopamine; NA, noradrenaline; 5-HT, Serotonin.

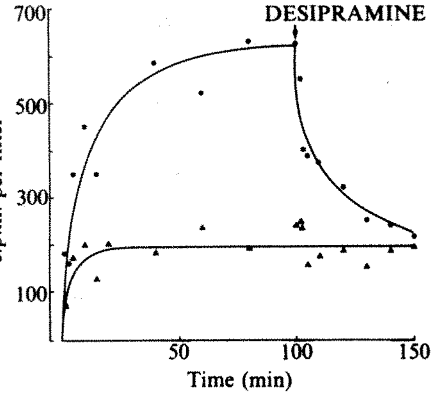

Fig. 2 Time course of ^{3}H-imipramine total (●) and nonspecific (▲) binding. 2.5 nM ^{3}H-imipramine was incubated with the membrane suspension at 0 °C as described in the text and samples were filtered at various times. At 100 min desipramine (10 μM) was added to displace the specifically bound ^{3}H-imipramine.

(Table 2) shows them to be unrelated. Desipramine, for example, which is at least 10 times more active than imipramine at inhibiting noradrenaline uptake and 10 times less active at inhibiting the uptake of serotonin, is almost equiactive with imipramine at inhibiting ^{3}H-imipramine binding. Although various other compounds were weakly active in inhibiting specific ^{3}H-imipramine binding (Table 1), there is no clear pattern or obvious relationship with other known pharmacological activities. Muscarinic and histaminergic H_2 receptors, which have been implicated in the action of tricyclic antidepressants,[8,9] do not seem to be involved, as atropine and cimetidine (muscarinic and H_2 histaminergic antagonists, respectively) are inactive.

It is difficult to establish a correlation between an *in vitro* biochemical activity of a drug and its clinical therapeutic efficacy in depression because of the heterogeneous nature of the clinical condition, the variable plasma levels resulting from the same dose and the similarity of potency for the commonly used antidepressants. Comparison of binding data with *in vivo* pharmacological tests which predict therapeutic drug activities is another possibility. The inhibition of reserpine-induced ptosis in mice is a widely used predictive test for antidepressant activity[14,15]. A comparison of the inhibition of reserpine-induced ptosis in mice with the inhibition of ^{3}H-imipramine binding in rats for a series of seven tricyclic and atypical antidepressants (imipramine, desipramine, amitriptyline, chloripramine, iprindole, viloxazine and mianserin) gave a correlation coefficient, *r*, of 0.588 ($P<0.25$) (R.R., M.B. and S.Z.L., unpublished results). A larger sample is, however, needed before any conclusions can be drawn. The poor correlation might be due to such factors as metabolic conversion to more or less active forms of the drugs and the possibility that tricyclic and atypical drugs really fall into two classes of which only the former act on the ^{3}H-imipramine binding site.

Thus, the specific binding of ^{3}H-imipramine to rat brain membranes is of high affinity, rapid, saturable and possesses a unique selectivity. It is, however, too early to say whether this binding is to an as yet undescribed receptor, as seems to be the case for the benzodiazepines. However, our results suggest that it may be related to the site of action of tricyclic antidepressant drugs, and as such could open up new approaches to the study of the biochemical basis of depression and its treatment with drugs.

A rapid communication of these findings has already been published[16].

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Attempted asymmetric radiolysis of D,L-tryptophan with ^{32}P β radiation

W. A. Bonner & N. E. Blair

Department of Chemistry, Stanford University, Stanford, California

J. J. Flores

Ames Research Center, NASA, Moffet Field, California

In 1957 Vester[1] suggested that the then recently discovered[2,3] violation of parity in weak interactions might be ultimately responsible for the present unique chirality of asymmetric molecules in the biosphere. Since then there has been a growing number of investigations involving the longitudinally polarised electrons from natural β decay. Initial attempts[4,5] to detect asymmetric effects in several synthetic and degradative organic reactions using a number of β emitters failed and it was not until 1968 that Garay reported[6] that β radiation from ^{90}Sr/^{90}Y caused D-tyrosine to degrade more than L-tyrosine in alkaline aqueous solution. Studies have subsequently looked at the possible asymmetric interaction of β rays (or their bremsstrahlung) from a variety of sources (for example ^{104}Rh (ref. 7), ^{90}Sr/^{90}Y (refs 8, 9), ^{14}C (refs 10–12) ^{32}P (ref. 13) and polarised electrons from an accelerator[14,15]) with a number of racemic or optically active substrates (usually amino acids). Conflicting results emerging from these and certain related experiments have been tabulated[13]. Perhaps the most striking positive report among these studies is the claim by Darge and coworkers[13] that D,L-tryptophan (4.9×10^{-7} mol in 2 ml frozen (−25°C) aqueous solution) was 33% decomposed with a 19% relative enrichment of the D-enantiomer in the undestroyed residue by the action during 12 weeks of the β rays from 5 mCi of co-dissolved ^{32}P phosphate. The 33% gross decomposition was estimated from the UV absorption spectrum, and the 19% optical enrichment from the optical activity of the crude diluted reaction mixture, as compared with L-tryptophan at the same concentration ($\sim10^{-5}$ M). The observed optical rotations used in the latter calculation were minute, −0.0036° for L-tryptophan and +0.0007° ± 0.0004° (average of 16 observations) for the crude diluted reaction mixture. Because the remarkably large asymmetric effect claimed was based solely on these small observed rotations, and as the results have already been used by others in additional calculations[16], we thought it desirable to attempt to duplicate the observations of Darge *et al.*[13]. We report here the use of a different analytical technique to estimate the enantiomeric composition of the undecomposed residual tryptophan, namely, gas chromatography (GC). The significant advantages of GC over optical rotation for determining the enantiomeric composition of small amounts of an optically active component admixed with impurities have already been described[17].

Two 2-ml aliquots of a solution of D,L-tryptophan (5.1 mg) in 1.8×10^{-3} M HCl (100 ml) were placed in culture tubes and each was treated with 5 mCi (200 µl) of aqueous $KH_2\,^{32}PO_4$ solution (NEN). A control for each sample was prepared by mixing 2 ml of the above tryptophan solution with 200 µl 0.042 M KH_2PO_4. The tubes were sealed and stored at −25 °C for 85 days, then thawed and opened. The irradiated samples were each divided into two equal portions and one portion was treated with 20 µl of a 1.3×10^{-2} M solution of D-tryptophan to allow estimation of the per cent degradation using GC and the enantiomeric marker technique[18]. Each solution was then warmed and evaporated in a nitrogen stream and the residues were treated with 1 ml 2-propanol saturated with HCl gas. The culture tubes were sealed and heated at 120 °C for 2.5 h, opened and the volatiles were again evaporated in a nitrogen stream. The residues were treated with 1 ml ethyl acetate and 50 µl heptafluorobutyric anhydride, then were maintained at 120°C for 45 min. The volatiles were again evaporated and each oily residue was dissolved in dichloromethane to give an $\sim10^{-2}$ M solution for GC analysis. The samples were analysed as previously described[17] using a 46 m × 0.5 mm (i.d.) stainless steel capillary GC column coated with *N*-docosanoyl-D-valine-*tert*-butylamide phase[19]. At 160 °C (isothermal) and a N_2 flow rate of ~10 ml min^{-1}, baseline GC resolution of the enantiomeric tryptophan derivatives was achieved with the L-isomer eluting in 51.7 min and the D-isomer in 56.9 min. All samples were analysed in replicate, with the control samples interspersed 'back-to-back' with the irradiated samples. The results of these analyses are shown in Table 1, along with the analysis of a known mixture of D- and L-tryptophan which was included to see if an enantiomeric excess (e.e.) as small as ~6% could be reliably detected with such tryptophan derivatives by the GC technique used. A combined GC–mass spectra analysis of the above D,L-tryptophan derivative showed that each GC peak gave the same fragmentation pattern and corresponded to a tryptophan isopropyl ester having one heptafluorobutyryl residue on each nitrogen atom.

Table 1

Exp	1*	1C†	2*	2C†	Known mixture‡
% Degradation	48	—	39	—	—
s.d. (±)	9	—	9	—	—
% D-tryptophan	50.5	51.1	49.5	50.8	48.3
% L-tryptophan	49.5	48.9	50.5	49.2	51.7
s.d. (±)[4]	0.6	0.2	0.1	0.3	0.7

* Irradiated samples of D,L-tryptophan.
† Control samples of D,L-tryptophan.
‡ Containing 47.1% D- and 52.9% L-tryptophan.
s.d. Standard deviation.

Although the gross degradation which we observed (43.5% average, Table 1) was somewhat larger than that reported (33%) by Darge *et al.*[13], we found no evidence whatsoever for any asymmetric radiolysis. While the GC analyses in Table 1 do not seem to be quite as precise as those previously noted[17] for leucine enantiomers, it is nevertheless clear that the irradiated tryptophan residues have an enantiomeric composition not experimentally distinguishable from that of the D,L-tryptophan controls. Furthermore, the detection of a known 5.8% e.e. (as 3.4%) in the known mixture of Table 1 suggests that the ~19% e.e. (59.7% D-, 40.3% L-tryptophan) claimed by Darge *et al.*[13] could scarcely have been missed by the present GC technique. Unable to find an asymmetric effect after using an alternative analytical procedure (GC), we can only conclude that Darge's reported 19% asymmetric radiolysis of D,L-tryptophan with ^{32}P β radiation may have resulted from a systematic error in the very small polarimetric readings involved or from an artefact present in the crude solutions examined.

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W. Darge*, I. Laczko† and W. Thiemann‡ reply: Bonner *et al.* criticise the reproducibility of our earlier results[1] and claim to have reproduced the original experiment. In fact they have applied a very different analytical technique based on gas-chromatographic analysis of the irradiation residue. There is also the difference that Bonner *et al.* have been able to analyse exclusively the enantiomeric composition of the partially destructed residual tryptophan only, while in our earlier optical rotatory dispersion (ORD) measurements we were able tc detect the effect of the sum of all potential chiral products (be they residual tryptophan, intermediate or final irradiation products such as any oxyacids, ketonic, or even peptidic substances commonly encountered in similar experiments). There are possibilities that any such intermediate products exhibit quite specifically large optical rotations and thus contribute to the overall effect in spite of their possibly small concentration in the investigated solution. We believe that this is exactly the advantage of a carefully performed ORD measurement over GC or other analytical methods. Having eliminated 'physical artefacts' as far as possible according to available techiques[2], one is left with any 'chemical artefacts' in the above defined sense, namely the existence of hitherto unidentified chiral intermediate irradiation products in the chain of the decomposition process of D,L-tryptophan. We never had claimed that any 'stereoselective effect' would manifest itself necessarily in the destruction of racemics but included the equally probable chance of stereoselective synthesis as a source of optical activity.

We wonder why Bonner *et al.* declare out earlier results as 'the most striking positive result': Garay in his original paper[3] and Merwitz[4] had both published much larger 'striking' stereoselective effects as a result of irradiation. Bonner *et al.* themselves confirm the basic hypothesis with their polarised electron bombardment of D,L-leucine[5]. What then is the essential difference between accelerated polarised electrons and β^- particles emitted from radionuclides in this context of 'helicity transferred from the classically physical level to the chemical level'?

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* Fachhochschule Aachen, FRG. † Biological Research Center Szeged, Hungary. ‡ Fachbereich Chemie, Universität Bremen, Postfach 330 440, D-2800 Bremen 33, FRG.

Evidence that three structural genes code for human alkaline phosphatases

Lorne E. Seargeant & Robert A. Stinson

Department of Pathology, Medical Laboratory Science, University of Alberta, Edmonton, Alberta, Canada T6G 2G3

The number of structural gene loci that code for the different molecular forms of human alkaline phosphatase is unknown. Physical properties of the enzymes, immunological data, chemical inhibition and genetic studies suggest that at least three structural genes[1] are involved: one coding for alkaline phosphatase from placenta, another for the enzyme from intestine, and one or more for the enzymes from liver, kidney and bone. Badger and Sussman[2] have shown that alkaline phosphatases from human liver and placenta are products of different structural genes, and Greene and Sussman[3] have shown that alkaline phosphatase from a metastasised bronchogenic carcinoma was nearly identical to the enzyme from placenta. However, other tumour-associated alkaline phosphatases and the enzymes from normal tissues other than placenta and liver have not been identified by conclusive structural criteria[4], and thus it is not known whether these onco-alkaline phosphatases represent ectopic production or unusual post-translational modification of the enzymes found in normal tissues. We present here, using a sensitive peptide-mapping technique[5], structural evidence that the enzyme forms from liver, kidney and serum from a patient with Paget's disease of bone (osteitis deformans) are products of the same structural gene and can be easily distinguished from either the intestinal or placental isoenzymes. The technique seems to be useful for the classification of tumour-associated alkaline phosphatases on a structural basis.

The relative pattern of radiolabelled peptides (Fig. 1) clearly shows that there are remarkable structural similarities between the enzymes purified from liver, kidney or Paget's serum. The maps of the radiolabelled peptides of alkaline phosphatase from intestine or placenta are markedly different from each other and from those for the other tissues. Products of different structural genes would be expected to show significant differences in amino acid sequences[11] and therefore have different tryptic–peptide compositions. But, allelic proteins of a single structural gene generally have almost identical amino acid sequences[11] and therefore would be more difficult to identify on the basis of tryptic–peptide composition. Thus our evidence strongly suggests that only three structural gene loci code for these alkaline phosphatases. The enzymes from intestine or placenta are products of two different loci, and the enzymes found in kidney, liver and in a serum from a patient with Paget's disease of bone, are products of a third locus.

The origin of the elevated serum alkaline phosphatase found in patients with Paget's disease of bone has not been proved, although it is generally considered to be derived from increased osteoblast activity[12]. The alkaline phosphatase level as determined by catalytic activity in the Paget's serum used in these studies was 30–40 times the level found in normal sera. The major component of the serum alkaline phosphatase was obtained in homogeneous form in 26% yield and was characterised as 'bone-type' alkaline phosphatase by heat stability, electrophoresis and differential inhibition studies.

Evidence that the stained protein bands were alkaline phosphatase protein was derived from experiments in which heating at 100 °C for 5 min was omitted when samples were prepared for SDS polyacrylamide gel electrophoresis (PAGE). The unheated samples in 1% SDS showed a single enzymatically active, slow-moving band which was coincident with the single protein-stained band[6]. When the protein is heated in 1% SDS in preparation for SDS-PAGE the enzymatically active band disappears and a faster migrating inactive band appears (equivalent to the subunit molecular weight) (Fig. 2). Peptide maps resulting from either treatment were identical. Also, the loss of enzymatic activity on prolonged incubation at 30 °C in the presence of 1% (w/v) SDS and 5% (v/v) mercaptoethanol was correlated with the disappearance of the slow-moving active band and the appearance of the faster inactive band in the SDS gels[6]. In addition, the peptide map of the protein band from analytical PAGE[13] of a liver alkaline phosphatase preparation was identical to that obtained by SDS-PAGE. We have also carried out chemical inhibition, electrophoretic, isoelectric focusing and heat stability studies on both the native and desialylated enzymes from each source (unpublished results). We found that these results are entirely consistent with the three structural gene loci hypothesis that is strongly supported by the peptide maps. Furthermore, the properties of the individual enzymes were as would be expected from the appropriate tissue source.

Note that, although the apparent subunit molecular weight (MW) values determined by SDS-PAGE may not be accurate, as the enzymes are glycoproteins and could migrate anomalously on polyacrylamide gels[14], the enzymes from liver, kidney, intestine and Paget's serum all had apparent subunit MWs of ~92,000. The enzyme from kidney was heterogeneous and had subunit MWs of ~96,000, 70,000 and 53,000. However the peptide maps from these three bands suggest that the two lower MW species may have been derived from the larger protein species and thus may represent partially degraded enzyme protein. This degradation may have occurred during the isolation procedure.

The relative spot intensity of two peptide maps from the same preparation is variable. This may be due, at least in part, to variation in the extent of radiolabelling with ^{125}I. It is likely that tyrosine residues are selectively iodinated, but diiodotyrosine derivatives as well as iodinated derivatives of phenylalanine and histidine may also be formed[15]. Thus peptides may be represented by more than one spot depending on the extent of iodination.

Alkaline phosphatase from various sources may differ in carbohydrate composition. However, the similarity of the peptide maps of alkaline phosphatase from liver, kidney and Paget's serum suggests that differences in carbohydrate composition, if present, have little influence on the resultant peptide map. Elder *et al.*[16] have suggested that the glycopeptides do not migrate in the apolar solvent system used here for thin-layer chromatography. Also our observation that the peptide maps for native and desialylated liver alkaline phosphatase were very similar suggests that the presence of carbohydrate does not greatly influence the results.

The approach used here to classify tissue alkaline phosphatases on a structural basis is suitable for the identification of the enzyme from normal and cancerous tissues. Maps of the radioiodinated peptides can readily be obtained in only a few days using less than 2 μg alkaline phosphatase protein. In addition, it is not necessary to purify the protein to homogeneity provided that it can be separated sufficiently from other proteins by SDS-PAGE (or other techniques) to allow excision of the stained protein band from the gel. Classification of alkaline phosphatases by structural composition will avoid the potential inaccuracies present when classifying gene products on the basis of heat stability, differential inhibition, immunological specificity, electrophoretic mobility or other functional properties.

Another technique used by others to study the structure of alkaline phosphatases is active site labelling with ^{32}P. Two-dimensional electrophoretic analysis of the labelled enzyme or one-dimensional electrophoresis of the labelled and partially trypsin degraded enzyme have been used to compare the enzymes from milk, placenta, liver, bone and intestine[17,18]. Active site peptides of alkaline phosphatase have been similarly studied[19]. However, this technique is usually applied to impure preparations and makes the assumption that alkaline phosphatase is the only protein that incorporates phosphate in the

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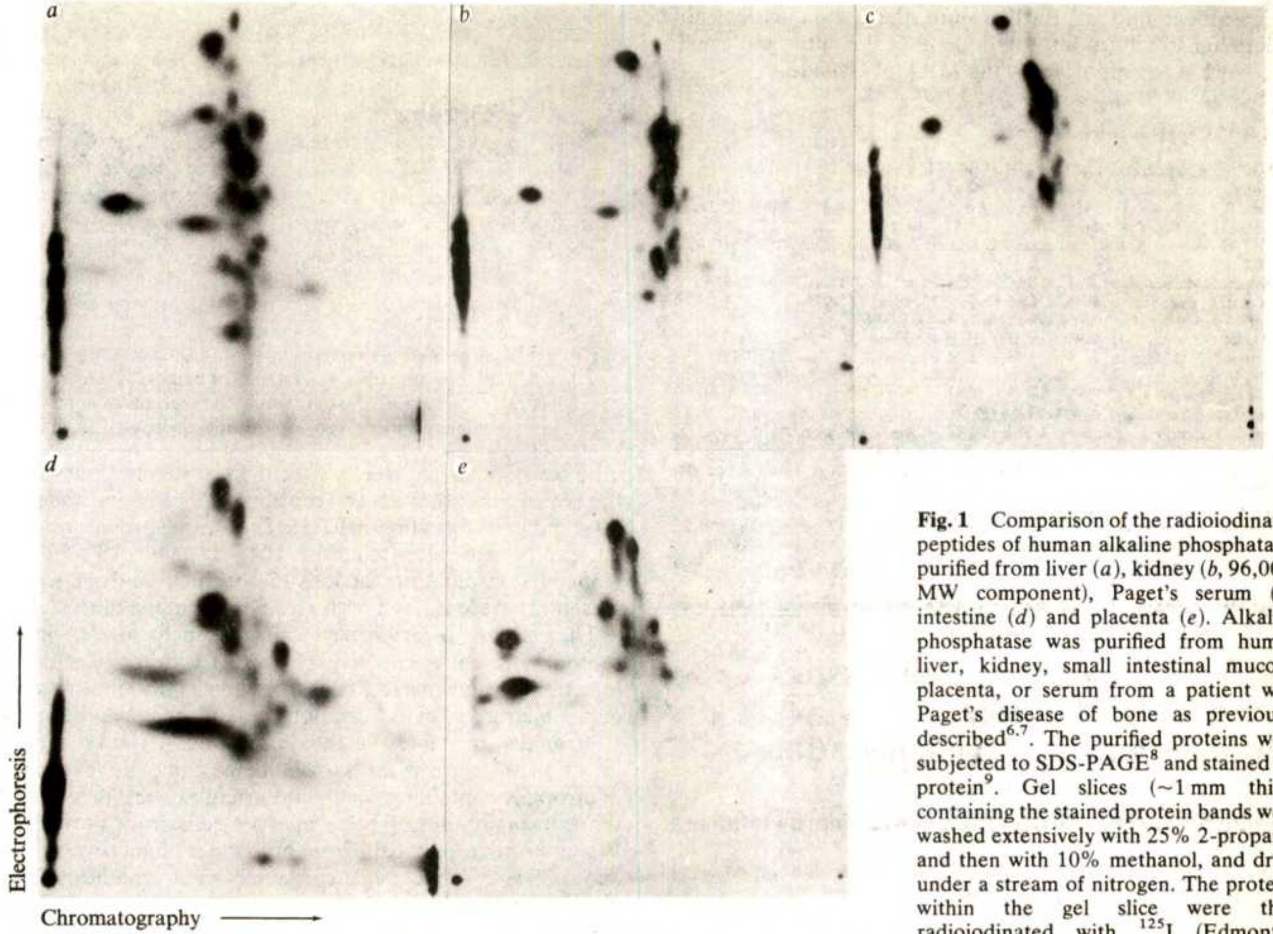

Fig. 1 Comparison of the radioiodinated peptides of human alkaline phosphatases purified from liver (*a*), kidney (*b*, 96,000-MW component), Paget's serum (*c*), intestine (*d*) and placenta (*e*). Alkaline phosphatase was purified from human liver, kidney, small intestinal mucosa, placenta, or serum from a patient with Paget's disease of bone as previously described[6,7]. The purified proteins were subjected to SDS-PAGE[8] and stained for protein[9]. Gel slices (~1 mm thick) containing the stained protein bands were washed extensively with 25% 2-propanol and then with 10% methanol, and dried under a stream of nitrogen. The proteins within the gel slice were then radioiodinated with ^{125}I (Edmonton Pharmaceutical, specific activity ~17 Ci mg^{-1}) by a modification of the chloramine *T* method[10] as described by Elder *et al.*[5]. Each gel slice was dried under a stream of nitrogen and incubated in 0.5 ml of 50 μg ml^{-1} trypsin (Sigma T-1005, 7,500 U mg^{-1}) in 50 mM NH_4HCO_3 buffer (*p*H 8.0) for 16 h at 37 °C, after which the supernatant was lyophilised. The tryptic digests were then analysed by thin-layer electrophoresis followed by thin-layer chromatography in a second direction. The lyophilised samples were dissolved in 20 μl of solution A (acetic acid/formic acid/water, 15:5:80) and ~1×10^6 c.p.m. (1–5 μl) were spotted onto 20 cm × 20 cm cellulose-coated TLC plates (Eastman Kodak). The plates were moistened with solution A and electrophoresed at 1,000 V for ~1 h at 8 °C. The plates were dried and chromatographed in a second direction using a solvent mixture containing butanol/pyridine/acetic acid: water (65:50:10:40). Radioactivity was detected by autoradiography using Kodak XR-1 X-ray film with an intensifying screen (Dupont, Cronex par speed) and exposure at −70 °C for 4 h, or as required.

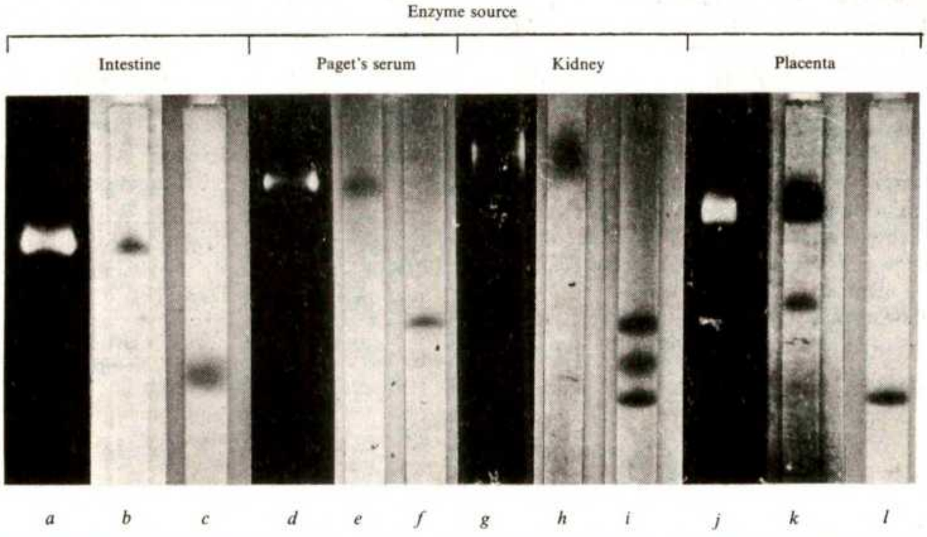

Fig. 2 Purity of alkaline phosphatases from human tissues. Alkaline phosphatases purified from small-intestinal mucosa, Paget's serum, kidney or placenta were subjected to SDS-PAGE[8] in denaturating and non-denaturing conditions. Identical criteria of purity for the enzyme from liver have been previously presented[6]. Non-denaturing conditions were identical to denaturing conditions except that heating the sample in 1% SDS to 100 °C for 5 min was omitted; instead, the sample in 1% SDS was left at room temperature for 5 min. *a, d, g, j*, Samples were prepared in non-denaturing conditions, electrophoresed, stained for enzyme activity[6], photographed and then (*b, e, h, k*) stained for protein[9]. *c, f, i, l*, Samples were prepared in denaturing conditions, electrophoresed and stained for protein[9]. Samples not denatured showed an enzymatically active band which corresponded to a protein-stained band. Two protein-stained bands were evident for nondenatured placental enzyme and both of these were active. In denaturing conditions samples from intestine, Paget's serum or placenta showed a single protein-stained band and the enzyme from kidney showed three bands (see text). For any one tissue denatured and non-denatured protein gave identical peptide maps. The two faster migrating bands in the kidney sample appeared to be proteolytic degradation products.

experimental conditions. Furthermore, the technique does not give detailed structural information about the entire molecule.

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Clonal nature of mast-cell clusters formed in W/W^v mice after bone marrow transplantation

Yukihiko Kitamura, Hiroshi Matsuda & Kaoru Hatanaka

Institute for Cancer Research and Department of Pathology, Medical School, Osaka University, Kita-ku, Osaka, 530 Japan

We have recently found that the number of mast cells in the skin of adult W/W^v mice is less than 1% of that observed in congeneic +/+ mice, and that no mast cells are detected in other tissues of W/W^v mice[1]. After the transplantation of bone marrow cells from congeneic +/+ mice, the number of mast cells in the skin, stomach, caecum and mesentery of the W/W^v mice increased to levels similar to those of the +/+ mice[1]. Study of the mast-cell number in the W/W^v mice at various times after transplantation suggested to us that mast cells might develop in groups, particularly in the skin and mesentery. In this report, we have attempted to elucidate the possible clonal origin of such mast-cell clusters from a single precursor cell, using giant granules of beige (C57BL/6-bg^J/bg^J, Chediak–Higashi syndrome) mice as a marker to identify the origin of the mast cells[2–4] (Fig. 1). We found that when WB-W/+ × C57BL/6-W^v (WBB6F$_1$)-W/W^v mice were injected with a mixture of bone marrow cells from beige C57BL/6 mice and normal C57BL/6 mice, more than 95% of mast-cell clusters consisted of either beige-type cells alone or normal-type cells alone. We conclude, therefore, that the cluster of mast cells originated from a single precursor cell.

Three-month-old WBB6F$_1$-W/W^v mice were injected with bone marrow cells from either beige C57BL/6 mice (2×10^7) or normal C57BL/6 (2×10^7), or with a mixture of bone marrow cells from beige C57BL/6 mice (10^7) and normal C57BL/6 mice (10^7).

On various days after the cell injection, a blood sample was obtained from a lateral tail vein of W/W^v recipient mice, and the number of erythrocytes was counted with a haemocytometer. The smears were fixed in formaldehyde vapour, stained with Sudan Black B and counterstained with Giemsa solution[5,6]. One hundred neutrophils with distinctly segmented nuclei were scored for the presence or absence of giant sudanophilic granules.

A biopsy of the dorsal skin of the W/W^v recipient mice was carried out 10 weeks after bone marrow transplantation, and the mice were killed 15 weeks after the transplantation. Pieces of the dorsal skin, stomach and caecum were removed and fixed in 10% buffered formalin (*p*H 7.2). The stretch preparation of the mesentery was also fixed in 10% formalin. Tissues were embedded in paraffin; sections (5 μm thick) and the stretched mesentery were stained with acidified toluidine blue.

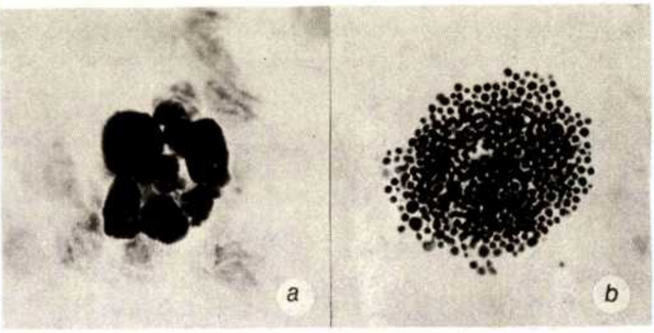

Fig. 1 Mast cells in a stretch preparation of mesentery, stained with acidified toluidine blue. *a*, Mast cell of beige C57BL/6 mouse; *b*, Mast cell of normal C57BL/6 mouse. The granules in the beige cell are significantly increased in size and decreased in number.

The number of erythrocytes increased to levels similar to those of normal mice (WBB6F$_1$-+/+) within 6 weeks of the injection of bone marrow cells from beige mice or normal mice, or the mixture of cells from both types of mice. About 80% of neutrophils contained giant granules 15 weeks after the injection of marrow cells from beige mice. In contrast, no neutrophils containing such giant granules were detected after the transplantation of bone marrow cells from normal mice. When the mixture of cells from both types of mice was injected, about 30% of neutrophils contained giant granules 15 weeks after transplantation.

Clusters of mast cells were detected in the sections of the skin removed by biopsy 10 weeks after the cell injection (Fig. 2*a*). All clusters consisted of mast cells containing giant granules alone when bone marrow cells of beige mice had been injected, whereas they were all composed of mast cells containing granules of regular size when the cells of normal mice had been used. When the mixture of cells from both types of mice was transplanted, about half of the clusters contained beige-type mast cells alone and the other half contained only normal-type mast cells. Although very few clusters were observed to contain both types of cells, one type of cell was predominant (more than 90% of total) in all cases. Serial sections of the skin revealed that the type of mast cells did not change in different parts of the same cluster.

In the skin, stomach and caecum of WBB6F$_1$-W/W^v mice killed 15 weeks after the injection of mixed marrow cells, it was

Table 1 Constitution of mast-cell clusters detected in the mesentery of WBB6F$_1$-W/W^v mice 15 weeks after the injection of a mixture of bone marrow cells from beige C57BL/6 mice and normal C57BL/6 mice

Mouse no.	No. of mast-cell clusters of each type			
	Pure beige	Pure normal	Mixed	Total
1	4	10	0	14
2	11	10	2	23
3	6	12	3	21
4	3	2	0	5
5	5	4	0	9
6	6	7	0	13
7	4	7	0	11
8	31	17	1	49
9	6	3	1	10
10	1	1	0	2
11	3	2	0	5
12	21	13	0	34
Total	101	88	7	196

The method of bone marrow transplantation has been described previously[1].

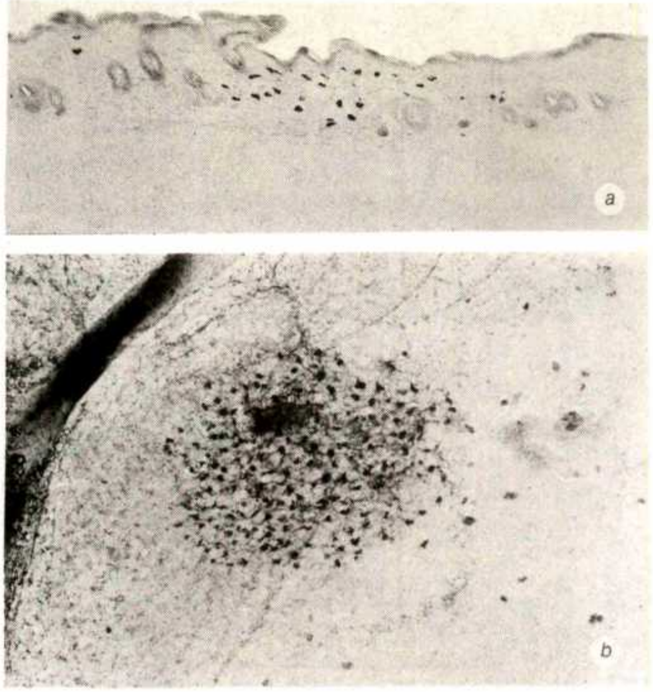

Fig. 2 *a*, A mast-cell cluster in the skin of a WBB6F$_1$-W/W^v mouse detected 10 weeks after bone marrow transplantation. A group of more than 10 mast cells in one section (5 μm thick) was defined as a cluster. *b*, A mast-cell cluster in the mesentery of a WBB6F$_1$-W/W^v mouse detected 15 weeks after the bone marrow transplantation. A discrete group of more than 20 mast cells in the stretched mesentery was defined as a cluster.

rather difficult to find discrete clusters because of the diffuse development of the mast cells. However, only one type of mast cell seemed to predominate in any given area of tissue.

In contrast with other tissues, discrete clusters of mast cells were observed in the stretched mesentery 15 weeks after bone marrow transplantation (Fig. 2*b*), located mainly in the fat tissue near blood vessels. As all cells contained in a cluster could be scored in the stretched mesentery without tedious examination of serial sections, the type and number of mast cells in each cluster were determined. As shown in Table 1, 189 out of 196 clusters (96%) consisted of either beige-type mast cells or normal-type mast cells. The largest cluster contained about 300 cells.

The present results demonstrate that mast-cell clusters which appeared in the skin and mesentery of W/W^v mice after the transplantation of bone marrow cells are derived from a single precursor cell. This is consistent with our recent observation that the precursor of the mast cell migrates through the bloodstream[7] and proliferates at the site of differentiation[4]. The bone marrow-derived precursor cell seems to produce up to 300 progenies when it differentiates into mast cells in the mesentery.

We have recently found that mast cells are as severely decreased in number in the Sl/Sl^d mice as in W/W^v mice[8]. In contrast to W/W^v mice, the number of mast cells in Sl/Sl^d mice did not increase after the transplantation of bone marrow cells from congeneic +/+ mice[8]. The injection of bone marrow cells from Sl/Sl^d mice increased the number of mast cells in the skin, stomach, caecum and mesentery of W/W^v mice. This indicates the presence of mast-cell precursors in the bone marrow of Sl/Sl^d mice[8]. The failure of haematopoietic colony formation in the spleen of Sl/Sl^d mice[9] and the failure of melanocyte differentiation in the skin of the same mutant mice[10] are attributed to a defect in the microenvironment[10–12]. The development of mast-cell clusters also seems to be under the control of the microenvironment.

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Interaction of C-reactive protein with artificial phosphatidylcholine bilayers

John E. Volanakis

Division of Clinical Immunology and Rheumatology, University of Alabama in Birmingham, Birmingham, Alabama 35294

Karel W. A. Wirtz

Laboratory of Biochemistry, University of Utrecht, Utrecht, The Netherlands

C-Reactive protein (CRP), the most characteristic of the 'acute phase proteins' (ref. 1) is thought to participate in the mediation and/or modulation of acute inflammatory processes, but its exact function is unknown. CRP has a Ca^{2+}-dependent binding specificity for phosphorylcholine,[2] the polar head group of two widely distributed lipids, lecithin (phosphatidylcholine, PC) and sphingomyelin (SM). A number of observations [3–5] suggest that at least some of the biological activities of CRP depend on its interaction with phospholipids of cell membranes. In addition, interaction of CRP with PC- and SM-containing lipid dispersions[6] and with PC-containing liposomes[7] can activate the complement system. We report here that binding of CRP to model membranes of PC requires the incorporation into the bilayer of lysophosphatidylcholine (LPC). Thus, a disturbance of the molecular organisation of the bilayer appears to be necessary for binding of CRP. These findings provide a possible biochemical explanation for binding of CRP to damaged but not intact cell membranes[3] and might be relevant to its biological function.

Binding of CRP to multilamellar PC-liposomes and single-walled PC-vesicles was studied by molecular sieve chromatography on agarose columns. CRP was purified from ascitic fluid by a recently described method[8] and labelled with ^{125}I by the lactoperoxidase method[9]. PC from egg yolk, LPC and phosphatidic acid (PA) were prepared as described previously [10]. Their purity was ascertained by thin layer chromatography on silica gel H. In initial experiments the binding of CRP to multilamellar liposomes and single-walled vesicles was examined. To this end, lipid dispersions consisting of PC, cholesterol and PA (77:15:8 mol%) were sonicated for short periods and after incubation with ^{125}I-CRP were subjected to chromatography on Sepharose-4B columns. As shown in Fig. 1, two peaks of phosopholipid were obtained: one containing large multi-layered liposomes corresponding to the void volume of the column, and a second containing small single-walled vesicles[11]. ^{125}I-CRP eluted from the column as a separate single peak at the same position as unbound protein. In addition, binding of ^{125}I-CRP was tested with neutral, negatively charged (10 mol% PA) and positively charged (10 mol% stearylamine) PC-vesicles by subjecting the CRP-vesicle mixture to chromatography on Bio-Gel A-0.5m columns. In repeated runs, the phospholipid and the protein eluted from the column as distinctly separate peaks. Only a small amount of the eluted

^{125}I-CRP (1–13%) was occasionally found associated with the vesicle peak. Identical results were obtained with dimyristoyl-PC and also when up to 40% cholesterol was incorporated into negatively charged vesicles. Similarly, when unlabelled CRP was substituted for ^{125}I-CRP only 10% of the eluted protein was associated with the vesicle peak. In these experiments, the concentration of CRP in the eluted fractions was determined by measuring intrinsic tryptophan fluorescence.

On the basis of these data we conclude that CRP cannot form a complex with the polar head group of PC present in an artificial bilayer. Given the known binding affinity of CRP for phosphorylcholine[12], these data could be interpreted to indicate that in intact PC bilayers the polar head groups are not freely accessible to the protein. This interpretation seems to contradict available models of the structure of phospholipid bilayers which indicate that polar head groups are exposed to the external aqueous phase and thus available for reaction. However, NMR data on artificial PC-bilayers have demonstrated the presence of intramolecular interactions between polar head groups restricting the extent of their accessibility to the water phase[13]. Furthermore, studies using human red cells revealed that hydrolysis of phosphoglycerides by phospholipase C required the presence of surfactants [14], indicating that the polar head groups were not freely accessible to this enzyme in the intact bilayer. These considerations led us to test the effect of detergent incorporation into the PC-bilayers on the binding of CRP. Considerable amounts of detergent can be incorporated into PC bilayers without disrupting them[15], and up to 50 mol% of LPC, Triton X-100 or SDS does not cause a breakdown of the bilayer structure. On the other hand, incorporation of detergent results in changes in a number of the known properties of the bilayers including changes in permeability[16], transition temperature[17] and susceptibility to hydrolysis by lipolytic enzymes[14].

The effect of incorporating increasing amounts of LPC into PC-vesicles on the binding of ^{125}I-CRP is shown in Fig. 2. At a molar ratio of PC:LPC 2:1, 83% of the eluted ^{125}I-CRP was found associated with the phospholipid peak. The ratio of radioactivity to lipid phosphorus was constant across the peak, indicating homogeneous binding of the protein to the vesicles. This shows that the membrane interface formed by PC and LPC exposes the phosphorylcholine group to the extent that CRP can form a regular complex. Increasing the molar ratio of PC:LPC resulted in a decrease of the percentage of vesicle-bound ^{125}I-CRP with a concomitant increase of the free ^{125}I-CRP peak. Thus, the percentage of eluted ^{125}I-CRP found associated with vesicles containing PC:LPC at molar ratios of 4:1, 10:1 and 1:0 (no LPC) was 73, 34 and 12%, respectively.

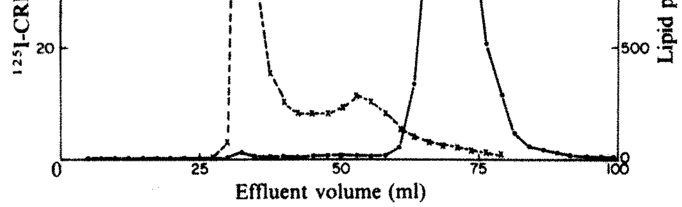

Fig. 1 Gel filtration of a mixture of ^{125}I-CRP and PC-vesicles using Sepharose 4B. Vesicles were prepared by suspending 55 μmol egg-PC, 11 μmol cholesterol and 5.5 μmol PA in 5.0 ml of 10 mM Tris-HCl, 150 mM NaCl, 2 mM $CaCl_2$, pH 7.2. The dispersion was subjected to sonic irradiation for 10 min at 0 °C under a stream of nitrogen, using a Branson tip sonifier at 70 W. Vesicles (6.6 μmol PC) were incubated with 0.56 nmol ^{125}I-CRP in a total volume of 0.8 ml for 30 min at room temperature. The mixture was then applied on a 1.5 × 45 cm column of Sepharose 4B equilibrated in 10 mM Tris, 150 mM NaCl, 2 mM $CaCl_2$, pH 7.2. Phospholipid content of fractions is expressed as lipid phosphorus, measured by the method of Rouser *et al.*[19]. The recovery of applied protein and lipid was 78% and 75% respectively.

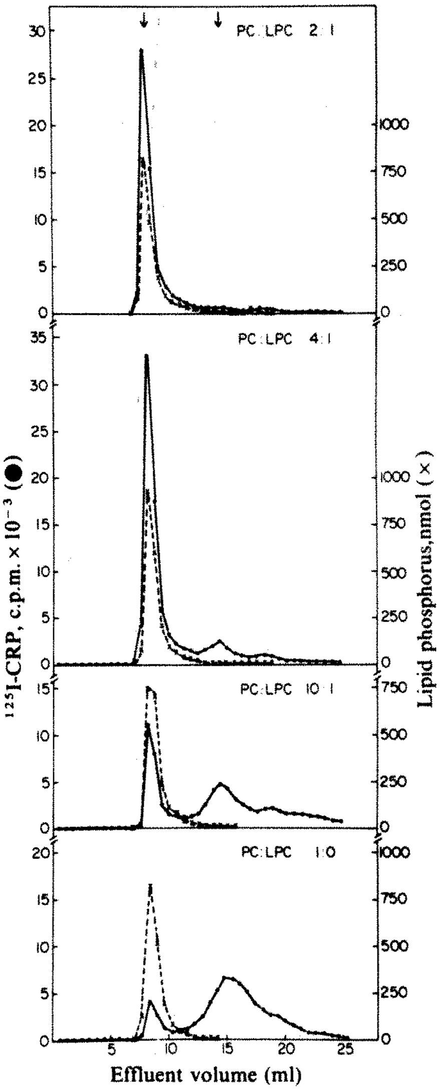

Fig. 2 Gel filtration of mixtures of ^{125}I-CRP and PC-vesicles containing various amounts of LPC, using Bio-Gel A-0.5 m. Vesicles were prepared from a chloroform solution of a mixture of egg-PC and egg-LPC at the indicated molar ratios. The lipid was dried by rotary evaporation under reduced pressure and dispersed in 10 mM Tris-HCl, 150 mM NaCl, pH 7.2 at a concentration of 12 μmol ml^{-1}. The dispersion was sonicated under nitrogen at 0 °C until it became clear. Sonication time varied from 10 min for the PC:LPC 2:1 vesicles to 45 min for the PC vesicles. The vesicle preparations were then centrifuged at 160,000g for 30 min to remove metal particles and large lipid aggregates. Vesicles (2.6 μmol of phospholipid) were incubated for 30 min at room temperature with 0.23 nmol ^{125}I-CRP in a total volume of 315 μl in buffer containing 9.5 mM $CaCl_2$. The mixture was then applied on a 7 × 45 cm Bio-Gel A-0.5 m column equilibrated and eluted with 10 mM Tris-HCl, 150 mM NaCl, 2.5 mM $CaCl_2$, pH 7.2. Mean recoveries ± s.e.m. for ^{125}I-CRP and phospholipid were 67 ± 4% and 79 ± 3%, respectively. The arrows indicate elution volumes of blue dextran (left) and ^{125}I-CRP (right).

To examine whether the observed binding of CRP to the vesicles was an exclusive function of LPC or could also be reproduced with other surfactants, three other surfactants were also examined. Figure 3 shows that Triton X-100 (Rohm and Hass), or sodium deoxycholate (DOC, Merck) incorporated into egg-PC-vesicles at molar concentrations of 45% did not promote the binding of ^{125}I-CRP to the vesicles. The experiment with Triton X-100 was repeated by equilibrating and eluting the column in buffer containing 0.35 mM Triton X-100, which is slightly above its critical micellar concentration, to avoid dissociation of the surfactant from the vesicles during the chromatography[18]. Again, no binding of ^{125}I-CRP to the vesicles was observed. However, when sodium dodecyl sulfate (SDS, Merck) was incorporated into PC-vesicles at the same molar concentration of 45%, some binding of ^{125}I-CRP was observed. As shown in Fig. 3, 38% of the eluted protein was associated with the SDS-containing vesicles. This compares to a binding of 84% for the PC-vesicles that contain LPC at a molar concentration of 50%.

The present data demonstrate that LPC is by far the most effective surfactant in promoting the binding of CRP to PC vesicles. We do not yet know whether binding occurs to the polar head group of LPC or to that of LPC and PC. The failure of Triton X-100 and DOC to promote binding raises the question of whether derangement of the molecular organisation of the PC bilayer is a prerequisite for binding. On the other hand, different surfactants are known to interact differently with phospholipid bilayers, the mode of interaction depending on such factors as the overall conformation, size, charge and length of acyl chain[15]. In terms of the possible biological implications of the present observation, previous data on the *in vivo* deposition of CRP are relevant. Kushner and Kaplan[3] reported that at experimental inflammatory sites CRP could be demonstrated in close association with membrane structures of altered or necrotic but not of normal cells. Thus, an alteration or complete breakdown of the natural orientation of cell membranes appeared to be necessary for *in vivo* binding of CRP, although the nature of the CRP-binding moieties was not determined. However, considering the two sets of data together, it seems reasonable to speculate that following tissue injury or necrosis, CRP can bind to phosphorylcholine-containing phospholipids of necrotic or altered cell membranes. As previously suggested[6], such binding could lead to complement activation and generation of chemotactic and opsonic factors, resulting in attraction of phagocytic cells and enhanced phagocytosis and thus contributing to the repair of the injured site. This hypothetical sequence of events should be considered as a possible biological function of CRP.

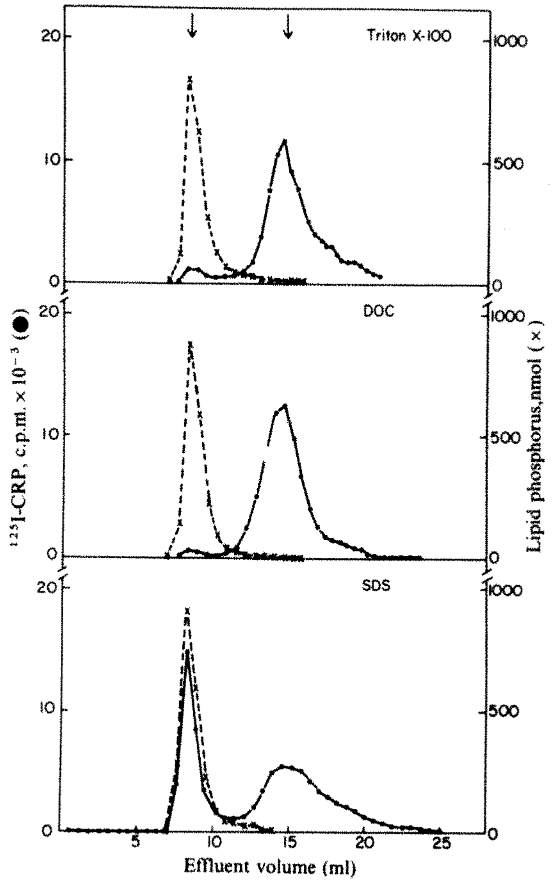

Fig. 3 Gel filtration of mixtures of ^{125}I-CRP and PC-vesicles containing various surfactants, using Bio-Gel A-0.5 m. Vesicles were prepared by dispersing egg-PC (11 μmol ml^{-1}) in 10 mM Tris-HCl, 150 mM NaCl, *p*H 7.2 containing 5 μmol ml^{-1} of the respective surfactant. Each dispersion was sonicated until it became clear (3–5 min). Other experimental details were as for Fig. 2. Mean recoveries ± s.e.m. for ^{125}I-CRP and phospholipid were 82 ± 4% and 88 ± 1%, respectively.

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Evidence for the bilobal nature of diferric rabbit plasma transferrin

B. Gorinsky, C. Horsburgh, P. F. Lindley, D. S. Moss, M. Parkar & J. L. Watson

Department of Crystallography, Birkbeck College, University of London, Malet Street, London WC1, UK

Plasma transferrin is involved in iron transport within the circulatory system of vertebrates, and provides an iron source for haemoglobin synthesis and other metabolic requirements. However, despite extensive studies by spectroscopic, biochemical and physiological techniques, the nature of iron binding and the mechanisms of uptake and release of iron are not fully understood. Plasma transferrins[1] are monomeric glycoproteins with a molecular weight of approximately 80,000 (ref. 2); they have two similar and very strong binding sites for Fe(III), together with two associated anion binding sites. Fragmentation studies on various transferrins[3-6] have shown that the polypeptide chain is composed of two domains formed from the N-terminal and C-terminal halves of the polypeptide chain. Each domain contains one metal binding site. The marked sequence similarities which exist between the two halves may reflect a doubling of an ancestral structural gene during the phylogenetic development of the protein[2,7]. Preliminary crystallographic investigations of diferric rabbit plasma transferrin have been reported from this laboratory[8]. We now report initial studies of the X-ray structure determination of diferric rabbit plasma transferrin which have led to a 6-Å resolution electron density map.

The protein crystallises in the space group $P4_12_12$ or the enantiomorph, with $a = b = 127.5(3)$ Å, $c = 145.4(3)$ Å, and one molecule per asymmetric unit. The native crystals are grown within the temperature range of 4–8 °C; at higher temperatures

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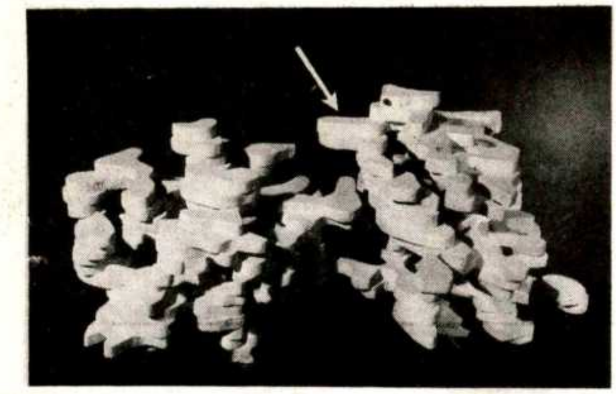

Fig. 1 A balsa wood model of electron density taken to represent one molecule of diferric rabbit plasma transferrin. The bilobal nature of the molecule is clearly seen and the arrow indicates the region of high electron density in the right hand lobe which may be α-helix. The prominent cleft in this lobe lies immediately below this region. The common platinum/mercury site is at the top of the left hand lobe.

they dissolve. Sixty-eight per cent of the crystal volume is occupied by solvent. The crystals are unstable in the absence of the mother liquor and derivatives were prepared by soaking the native crystals in solutions of heavy atom reagents in the mother liquor. The soaking conditions for the three derivatives used to produce the map are given in Table 1.

X-ray diffraction data for an octant of reciprocal space were collected on a Hilger and Watts Y290 automated four-circle diffractometer for the native crystals and three isomorphous derivatives. The diffractometer was equipped with a helium path, and a low-temperature device and Ni-filtered copper radiation were used throughout. Data were collected at 5 °C using ω-step scans. Three or four crystals were required per data set and intensities were estimated by a 'moving window' technique[9]; absorption corrections were made according to the method of North *et al.*[10].

Difference Patterson syntheses computed with ΔF_{iso}^2 (where ΔF_{iso} is the isomorphous difference) as coefficients enabled two major heavy atom sites to be located for the mercury derivative and one site for the platinum derivative. Only a partial interpretation of the uranyl map was possible at this stage. The three major uranyl sites were located from difference maps computed with protein phases obtained by multiple isomorphous replacement using the refined mercury and platinum positions. In addition, the heavy atom positions of the mercury and platinum derivatives were checked by the use of cross-difference Fourier syntheses based on single isomorphous protein phases from the uranium positions.

For each derivative the heavy atom coordinates and relative occupancies were refined against ΔF_{iso} for the centric reflections. Isotropic temperature factors were held constant at a value of 20 Å. Difference electron density maps were computed with coefficients $(\Delta F_{iso} - F_{Hcalc})e^{i\alpha_{Hcalc}}$ (where F_{Hcalc} and α_{Hcalc} are the calculated amplitude and phase of the heavy atom structure factor), but only revealed significant minor sites for the uranyl derivative.

The final heavy atom parameters and R factors are presented in Table 2. Protein phases were calculated from these parameters using the method of multiple isomorphous replacement with all three derivatives in space group $P4_12_12$.

Table 1 Derivatives used to produce the electron density map

Heavy-atom reagent	Time of soaking	Concentration	Method
Potassium chloroplatinate	36 h	7 mM	Direct soaking
Mercuric chloride	24 h	8 mM	Dialysis cell
Uranyl acetate	9 d	10 mM	Dialysis cell

Data collected to 6 Å.

Table 2 Heavy atom parameters and refinement statistics

Site	x	y	z	Z	B(Å^2)
Mercuric chloride					
1	0.487	0.125	0.178	0.086	20
2	0.032	0.278	0.185	0.046	20
		R(centric) = 0.61			
Potassium chloroplatinate					
1	0.024	0.285	0.184	0.063	20
		R(centric) = 0.58			
Uranyl acetate					
1	0.307	0.257	0.090	0.032	20
2	0.214	0.495	0.090	0.029	20
3	0.633	0.391	0.013	0.020	20
4	0.756	0.066	0.069	0.010	20
5	0.458	0.315	0.075	0.014	20
		R(centric) = 0.62			

x, y, z are the heavy atom fractional co-ordinates. Z is relative occupancy. All site occupancies are on the same arbitrary scale. $\langle|\alpha_P - \alpha_H|\rangle$, the mean absolute difference between the protein and heavy atom phase angles, is 86.9°, 89.3° and 90.7°, respectively, for mercuric chloride, potassium chloroplatinate and uranyl acetate.

The mean overall figure of merit for the 3,226 reflections is 0.50.

The 'best' phases and figures of merit[11] were used to calculate a 6-Å resolution electron density map. This map shows a well defined volume of high electron density with approximate maximum dimensions of 95 × 60 × 50 Å. This is represented by a balsa wood model (Fig. 1) which defines the molecular boundary. The high solvent content of the crystals and the low salt concentration of the liquid of crystallisation proved useful in this respect. The model shows that the molecule consists of two lobes of roughly equal size. The major axes of these lobes are inclined at about 30° to one another and their total volume is consistent with a protein content of about 30% in the crystal. All the heavy atom sites lie close to the surface of the molecule. Four of the uranyl sites and the major mercury site are on one lobe, and the remaining uranyl site and the common platinum and mercury site, which may correspond to the position of a methionine residue, are on the second lobe. The former lobe exhibits a pronounced cleft in the region of the join between the two lobes and close to this feature there is a column of high electron density which could be α-helix. On the opposite side of this same lobe another strong region of electron density may prove to be β-sheet at higher resolution. The second lobe also has a cleft, although less pronounced, close to the join.

The two lobes of the molecule may correspond to the two domains, each containing an iron binding site, proposed from fragmentation studies. However, the locations of the iron binding sites and the arrangement of amino acid residues in the diferric rabbit plasma transferrin molecule must await study at higher resolution. The collection of X-ray data to 3.0 Å resolution, the practical limit of the diffraction pattern using conventional sources, is now in progress.

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Advanced techniques in routine use at The Radiochemical Centre

Described below are just two examples of the many up-to-date techniques, which have been pioneered or applied for routine use at The Radiochemical Centre. These developments are part of our constant endeavour to maintain our position at the forefront of the specialised field of tracer methodology, so that we can continue our supply of radiochemicals of the highest quality and technical specifications.

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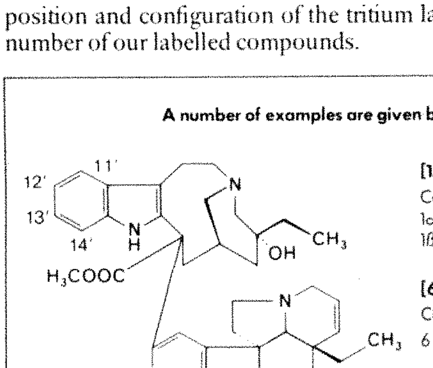
Modern techniques for the production of tritiated compounds are more sophisticated than those used in the early days of tritium labelling, and produce compounds labelled in specific positions rather than generally labelled. Nevertheless, it is necessary for many tracer applications of tritium compounds to know the *precise* position and configuration of the tritium labels. Traditional chemical methods of doing this are tedious and time consuming and subject to considerable error, and so the routine supply of such information has until recently not been possible.

The Radiochemical Centre, in collaboration with the University of Surrey, has developed over the past eight years the technique of tritium nuclear magnetic resonance (tnmr) spectroscopy for this purpose. This method is much quicker and more accurate than the traditional chemical or biochemical methods for determining distribution of tritium labelling.

It is now used routinely to establish the distribution of tritium labelling produced by the usual methods of tritiation employed at The Radiochemical Centre. We supply accurate details as to the position and configuration of the tritium labels for an increasing number of our labelled compounds.

A number of examples are given below:



[G-^{3}H]VINBLASTINE
Code TRK.507
17 .... 44% 11' 8%
12',13' .... 46% 14' 2%

[1β, 2β(n)-^{3}H]TESTOSTERONE
Code TRK.162
1α .... 5.9% 2α 8.2%
1β .... 46.0% 2β 39.9%

[6-^{3}H]BENZO[a]PYRENE
Code TRK.501
6 .... >95% 1 .... <5%

D-[2-^{3}H]GLUCOSE
Code TRK.361
2(α) .... 40% 2(β) 60%

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Code TRA/K.533
2 .... 8% 4 .... 64%
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AL-RAWI, J.M.A., BLOXSIDE, J.P., ELVIDGE, J.A., JONES, J.R., CHAMBERS, V.E.M., CHAMBERS, V.M.A. and EVANS, E.A., *Steroids* vol. 28 (3), p.p. 359-375, 1976.

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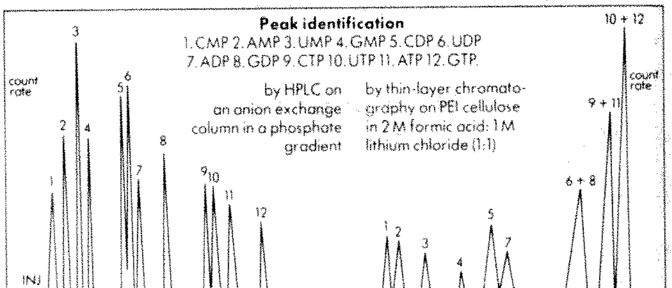
The example illustrated below illustrates the clear superiority of HPLC when used as an analytical tool. The mixture used comprised the tritium labelled mono-, di- and triphosphates of adenosine, cytidine, guanosine and uridine, and all are clearly separated in the HPLC system.



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reviews

Shaping psychology

O. L. Zangwill

Pioneers of Psychology. By R. E. Fancher. Pp. 397. (W. W. Norton: New York, 1979.) $15.95.

THE author tells us in his Preface that this book is about "the lives and works of some of the people who have shaped the modern science of psychology." Although there are still some people who hold, with William James, that psychology is less a science than the hope of a science, it can safely be said that the people Dr Fancher writes about, whether they were primarily philosophers or physicists, physiologists or physicians, have had much to do with the shaping of psychology as we know it today. His account even extends to a few, such as Wilhelm Wundt, who were essentially experimental psychologists even if the posts they occupied fell within other academic disciplines, and to two contemporary "pioneers", Jean Piaget and B. F. Skinner. Nevertheless, it is essentially an outline, somewhat superficial though extremely readable, of the background of modern psychology.

The opening chapter, on Descartes, sets the tone of the book. This may be described as a blend of thumb-nail biography and the history of ideas. As Dr Fancher right points out, Descartes' knowledge of anatomy was amateur even by the standards of his time and his physiology was mostly wrong. Nevertheless, his concept of reflex action and the mechanistic model of the nervous system which it inspired has largely animated the growth of neurophysiology and provided experimental psychology with its basic philosophy. True, Descartes' resolution of the mind-body problem has left many people unsatisfied but it has nevertheless remained an influential belief not only among philosophers but many scientists too. Cartesian dualism may well be wrong but it is inseparable from the evolution of modern science.

The next chapter is headed "The Physiology of Mind" and traces the development of our ideas about brain function from Gall's phrenology to present-day neurosurgery. Inevitably, Dr Francher deals at some length with Pierre Flourens, whose work on the effects of brain lesions on behaviour in animals did much to discredit phrenology and promote the view that sensory and motor functions lack discrete representation in the cerebral cortex. As has often been pointed out, his conclusions in many respects anticipated those of Karl Lashley over a century later. Indeed it was the clinicians rather than the experimental physiologists who changed the whole climate of localisation theory. Following the claims of Broca and others that supposedly circumscribed disorders of language might follow localised cerebral lesions, a kind of neo-phrenology evolved envisaged in terms not of bumps but of brain centres. Although the concept of a brain centre was soundly based on the theory of reflex action, it became only too readily contaminated by psychology. The result was a new mythology of cerebral functions bearing little relation either to the mechanisms of nervous action or to the realities of human behaviour.

It can perhaps be said that neurology is now working towards a less extreme conception of the cerebral localisation of psychological function. Although focal deficits resulting from cortical lesions have been charted with a very fair degree of precision, we still lack any clear conception of the relation of deficit to normal function and the extent to which the machinery of the brain may be inferred from studies of its breakdown. Interestingly, Dr Fancher views Penfield's work on the effects of cortical stimulation in conscious man as the latest and most decisive phase in the localisation controversy. Brilliant indeed as was Penfield's work, it cannot really be said to have made a major theoretical contribution to solution of the mind-body problem. Indeed, Roger Sperry's work on 'split-brain' man might seem to offer greater hope of a fresh consideration of the physical basis of mind and consciousness. It is a pity that Sperry's work fails to find mention in Dr Fancher's book.

We then come to two chapters mainly concerned with the origins of modern experimental psychology. The first deals principally with studies of human sensation and perception and centres on Helmholtz: the second with Wilhelm-Wundt, whom the author places in sharp contrast with William James, somewhat to Wundt's disadvantage. In connection with Helmholtz, Dr Fancher does well to place stress on the Kantian influence on his thinking, which his strongly empiricist bias has caused many to disregard. It is evident, for example, that, although Helmholtz laid great emphasis on the role of experience and learning in human perception, more especially the perception of space, he treated sensation as basically innate. Whether we regard Helmholtz as a physicist, a physiologist or a psychologist is irrelevant. Though he did not himself wish to be regarded as a psychologist, there can be no doubt that, without him, human experimental psychology would never have found its feet.

This inevitably brings us to Wundt, who is remembered as the founder of the first laboratory in the world exclusively dedicated to experimental psychology. Indeed it celebrates its centenary this year. James was sceptical as to its promise: As he tartly remarked, "there is little of the grand style about these new prism, pendulum and chronograph philosophers." Yet, to give him his due, Wundt did try to bring together psychophysical studies and the physiology of the senses, and his work enabled experimental psychology to emerge as an independent discipline. Yet James has perhaps been proved right in so far as experimental psychology, though nowadays respectable, is seldom scintillating.

The scene now shifts to a very different theme, remote from the University but close to the consulting room. This is the theme of psychological medicine, and its origins are traced to the early hypnotists beginning with Mesmer and culminating in Freud. On Mesmer, Dr Fancher has disappointingly little new to tell us. In particular, he throws no light on Pattie's contention that his doctoral thesis was cribbed almost word-by-word from a thesis by one Richard Mead, an obscure Irish physician. This claim, if true, detracts considerably from the more sympathetic judgements of Mesmer's personal integrity advanced by several recent biographers. On Freud, the subject of an earlier book by the same author, Dr Fancher is knowledgeable and sympathetic. His chapter is of considerable value in stressing the importance of much of Freud's earlier work, in particular his draft project for a scientific psychology, written in 1895 but published only several years after his death, and the early case studies on hysteria. His book on *The Interpretation of Dreams* rightly receives high commendation. It is disappointing, however, that Dr Fancher sees no obvious way whereby the insights of psychoanalysis might be linked with the sterner disciplines of the neurosciences.

The only reference to British psychology is a chapter on Francis Galton and the psychology of individual differences. This once again covers familiar ground and it is a pity that a recent biography by D.W. Forrest, altogether more digestible than Pearson's monumental three volumes, fails to find mention. Fancher's short account is useful in stressing Galton's fascinating studies of visual imagery and word-association, which have been relatively neglected in recent years, and he deserves credit for not getting too embroiled in the nature-nurture controversy, so productive of emotion though hardly of wisdom in contemporary psychological discussion.

The last two chapters bring us closer to the modern scene. The penultimate chapter gives an account of Pavlov's work followed by a short outline of the history of behaviourism. Although the account of J. B. Watson's life and work is not without historical interest, it fails to bring out fully how little behaviourism, in its early days at least, owed to Pavlov (it was more influenced by his rival Becterev); nor how greatly Pavlov himself detested behaviourism, which he regarded as little better than the mentalistic psychology it replaced. The importance of Lashley as a co-founder of behaviourism is insufficiently stressed. The final chapter contrasts Jean Piaget with B. F. Skinner, both described as modern pioneers. Only the future will show.

Although much of this book has been compiled from secondary sources and makes no advance on the widely accepted version of the history of experimental psychology which we owe to the late E. G. Boring, this book can be recommended for its thoughtful and well-written account of some important threads in its more recent phases of development. It is only perhaps in the sections dealing with German psychology that the author fails in some measure to catch the spirit of the age, due to the probability that he is unable to read German. Although it may be true that English is now the accepted language of European science, the historian of science would do well to acquire at all events, a reading knowledge of the mother tongue of European scientists. □

O. L. Zangwill is Professor of Experimental Psychology at the University of Cambridge, UK.

Free radical chemistry

Radicals. By D.C. Nonhebel, J.M. Tedder and J.C. Walton. Pp. 200. (Cambridge University Press: Cambridge, 1979.) Hardback £14; paperback £5.50.

To cover adequately the many important aspects of free radical chemistry in approximately 200 pages, even at the undergraduate level, is an impossible task. This book makes an excellent attempt at the impossible.

The book is aimed at honours and post-graduate chemists but should also be useful to chemists at all levels in need of background information on radical chemistry. The coverage is wide-ranging although the depth of treatment is variable. Almost no original references are quoted but there is an extensive list of books and reviews for each chapter collected at the end of the book.

Not unexpectedly, in view of the authors' research interests, the chapters entitled "Radical transfer reactions" (18 pages), and "Radical addition reactions" (22 pages) are particularly well done and are a first-class source of facts and current concepts of radical mechanisms. The chapter on "Radicals in biological systems" (15 pages) is also admirable and timely, as this is a growth area in free radical chemistry. Indeed, I would have preferred if this chapter had been somewhat longer, perhaps at the expense of part of that on "Radical polymerisation" (13 pages). The chapter devoted to "Stereochemistry of free radicals" (9 pages) is also most welcome, as this subject usually receives little attention in textbooks at this level. Awareness of the shape and preferred conformations of radicals is essential if a proper understanding of radical reactions is to be achieved.

Oxidation and reduction in free radical chemistry is a notoriously difficult subject to cover because the terms themselves seem to mean different things to different chemists and the subject matter does not lend itself easily to uniform treatment. Because of this the 15-page chapter on this subject seemed rather short and I would have preferred to have seen a fuller account given.

The book also gives brief introductions to the use of electron spin resonance spectroscopy and CIDNP, and there are chapters on combustion, radical reactions, homolytic aromatic substitution, radical rearrangements, and radical displacement reactions. All of these are sound, although perhaps not quite as good as some of the chapters mentioned earlier.

In summary, I can fully recommend this book as a compact account of present-day free radical chemistry. My criticisms of it are minor and are essentially restricted to the space allocation given to the various subjects.

A.R. Forrester

A.R. Forrester is Senior Lecturer in Chemistry at the University of Aberdeen, UK.

Reptilian ear

The Reptile Ear: Its Structure and Function. By Ernest Glen Wever. Pp. 1024. (Princeton University Press: Princeton, New Jersey, and Guildford, UK, 1979.) $50; £31.30.

The Reptile Ear is a monumental work of outstanding importance, representing more than 25 years of effort by a distinguished scientist. Much of the work described in this book has not been published before.

Detailed studies of the external, middle, and internal ears of 247 species of reptiles were investigated, including 186 species of lizards belonging to 16 families; 19 species of snakes; 14 species of amphisbaenians; 24 species of turtles; 3 species of crocodilians; and the single existing species of rhynchocephalians.

Wever's investigations were both anatomical and physiological, and this greatly enhances the significance of this work. The anatomical studies were carried out by means of serial celloidin sections. It is remarkable that Wever achieved such excellent and accurate detail by a light microscopic method, as his structural interpretations have been corroborated by scanning electron microscopic studies made by other workers.

Wever presents all his anatomical findings by drawings of excellent quality. It would have been helpful to others, however, to have at least some sections represented by photomicrographs.

A great deal of quantitative structural information is presented, such as papilla basilaris lengths, widths, hair cell numbers, basilar membrane width, numbers of rows of hair cells, and so on. Of particular interest is Wever's descriptions and classifications of various types of tectorial membranes.

Wever's evaluation of the functional capacity of these many ears is by use of the cochlear potential method. In many cases, Wever demonstrates increased functional capacity to be related to structural complexities. Such, however, is not always the case.

A great deal of attention is also directed to discussions of the mechanics of hair cell excitation, a problem of salient importance and interest at the moment. The concluding chapter on the biological and evolutionary significance of the reptilian ear is somewhat brief and perhaps properly conservative. Throughout the text, however, Wever indicates structure in relation to phylogeny and evolution. That the reptiles "experiment" widely with many ear components has been well demonstrated and is one of the reasons for the great interest in the reptilian ear.

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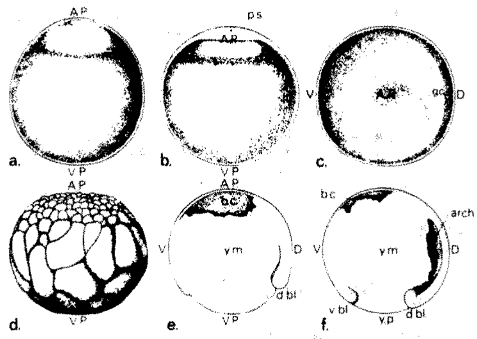

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The author has been careful to describe his working methods in great detail. This information should be of great value ot others.

Numerous recent studies have proved the great value of the reptilian ear as an object of study for investigation of the vertebrate auditory system. Wever's compendium will serve as a valuable and necessary reference and source book for future investigations. **Malcolm R. Miller**

Malcolm R. Miller is Professor in the Department of Anatomy, University of California School of Medicine, San Francisco, California.

African mammalogy

Ecology of African Mammals. By M.J. Delany and D.C.D. Happold. Pp. 434. (Longman: London and New York, 1979.) £25.

THIS book, the third contribution to Longman's Tropical Ecology Series covers Africa south of the Sahara. All aspects of the ecology of African mammals, both large and small, are considered resulting in a big book full of concentrated information. The tendency to categorise the text into sections and sub-sections does not make for easy reading but the approach will no doubt be appreciated by students wishing to note essential points. The book is divided into four parts and consists of thirteen chapters. The first part gives a comprehensive account of African mammals in an historical setting and the second concerns the biotic zones dealing in turn with rain forests, savannas, arid zones and mountains. The third part considers life histories, behavioural ecology, physiology and population dynamics; and the fourth section, consisting of only one chapter, examines the interactions between mammals and man.

The book, which is extremely well illustrated, covers the ground admirably and the authors have made a very thorough job of their survey of the literature. They have tried to give a balanced account, which is not an easy task considering that the coverage of the field by research workers is far from even. On the whole, they have selected wisely and have not been biased by their own particular research interests, which are mainly in small mammals. Their account of the large mammals, being essentially a literature review, inevitably lacks something of the critical insight which an active worker in the field would provide. A few of the examples could have been better chosen, for example Rwenzori National Park (p. 113) is not very representative of its region in terms of the number of large mammal species in view of the fact that six more ungulate species occur in a game reserve less than 100 km to the east. It is also unfortunate that the work of Dasmann and Mossman, now some 20 years old, should be quoted in support of game ranching because the yields proposed were hopelessly optimistic and could not have been sustained for long.

There are few errors in this excellent book and none of great significance. It will prove extremely useful as a university text particularly in Africa but also in Europe and America for, given the rich mammalian fauna of Africa, it can serve as a general textbook of mammalogy. Research workers will also find it of value as a reference book. **S.K. Eltringham**

S.K. Eltringham, formerly Chief Research Officer of the Uganda National Parks, is now Lecturer in Applied Biology at the University of Cambridge, UK.

Modern ionospheric physics

Inospheric Techniques and Phenomena. By A. Giraud and M. Petit. Pp. 264. (D. Reidel:Dordrecht, The Netherlands, Boston and London, 1978.) Dfl.85; $38.

IONOSPHERIC physics has rapidly evolved after the International Geophysical Year (1957-58), from the old days during which traditional ionospheric sounders were the main tool, to the new era during which a number of new powerful tools have been introduced to measure basic ionospheric quantities. As one of those who is aware of such progress and yet has difficulty in catching up on modern literature as a non-specialist in this particular field, I am delighted to have this book. It presents an overall view of the new ionospheric physics. In particular, the ionosphere is properly and accuratley recognized as an integral part of the solar-terrestrial system. In this sense, I feel that the title *Modern Ionospheric Physics* would have been more appropriate.

The book provides a well-balanced presentation of the subject, and will serve as a good textbook for a graduate level course in aeronomy as well as an important reference book for ionospheric physicists and magnetospheric physicists. The book will familiarise readers with all modern techniques in investigating the ionosphere, equipping them well in interpreting data taken from such techniques and also in understanding very recent and future articles in this particular field.

Syun-Ichi Akasofu

Syun-Ichi Akasofu is Professor of Geophysics at the Geophysical Institute, University of Alaska, Fairbanks, Alaska.

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Ocean yearbook

Ocean Yearbook 1. Edited by Elisabeth Mann Borgese and Norton Ginsburg. Pp. 890. (Pacem in Maribus/International Ocean Institute/University of Chicago Press: Chicago and London, 1979.) £17.50; $25.

ELISABETH Mann Borgese, International Ocean Institute, Malta, and Norton Ginsburg, University of Chicago, are to be congratulated on getting their latest brainchild, the *Ocean Yearbook*, off the ground with its first edition, recently published. Although named a yearbook, the year in question has not been identified; however, the reports of the various organisations which appear in an Annex, cover 1976 and, in certain cases, to mid-1977.

Following a short Editors' Preface, *Ocean Yearbook 1* contains a series of excellent topical articles on Issues and Prospects; Living Resources; Non-living Resources; Transportation and Communication; Marine Science and Technology; Environment; Coastal Management; Military Activities; and Regional Development. These are followed by a number of appendices covering reports from organisations, selected documents and proceedings, a directory of institutions, tables related to the articles, and an index.

The *Yearbook* is a long-awaited product of the International Ocean Institute, Malta, which is described in the Preface as "an offspring of an initiative of the Center for the Study of Democratic Institutions in Santa Barbara, California, USA". Publication of an Ocean Yearbook is the logical outcome of the present greatly increased interest in the Oceans which has been kindled by the Third United Nations Conference on the Law of the Sea. It is therefore vitally necessary to follow the developments which are taking place at the Conference, as presented in an excellent critique of the Informal Composite Negotiating Text (1977) by Arvid Pardo, who, in 1967, speaking as the Ambassador of Malta to the United Nations General Assembly, was originally responsible for drawing the attention of the world's governments to the potential of the oceans and their importance to the future of mankind.

Although the key to most other activities in the oceans, there is only one article on Marine Science (and Technology), Perspectives on the Sciences of the Sea by Lord Ritchie-Calder, who sounds a well-justified note of warning in his final two paragraphs. As stated by Sir Francis Beaufort, Hydrographer of the Navy (1829-1855), "The natural tendency of men is to undervalue what they cannot understand". The editors, by their imbalance of content in favour of applied activities at the expense of basic science, are giving credence to this unfortunate tendency which is particularly prevalent today in the United Nations system and amongst the national aid agencies.

I would also suggest that the selected Documents and Proceedings, contained in Appendix B, should be restricted to "a selection of international agreements, legislation and proceedings of international conferences" which have been finalised. Despite its relevance to Arvid Pardo's article, I query the desirability of reproducing in full the UNCLOS Informal Composite Negotiating Text which had already been replaced by a revised edition before the Yearbook was published.

The errors I have noted are minimal. This is a well compiled and well edited publication. My main fear is that the editors have set their sights too high and will be unable to maintain their present standard and the sheer volume of information contained in the first edition; in any case, at yearly intervals as its name implies. I note only that in the bibliography to Lord Ritchie-Calder's article, Frye, P. is presumably Paul M. Fye, now President of the Corporation, Woods Hole Oceanographic Institution. I must also inform Tom Busha and James Dawson that the only flaw I can find in their most informative article on that excellent organisation IMCO is that the Hydrographic Department of the Admiralty was founded in 1795 (not 1759) when Alexander Dalrymple, Hydrographer to the East India Company, was appointed the first Hydrographer of the Navy.

Desmond P.D. Scott

Desmond P.D. Scott is Secretary of the Intergovernmental Oceanographic Commission, Unesco, Paris.

Magnetohydrodynamic stability

MHD Instabilities. By G. Bateman. Pp. 263. (MIT Press: Cambridge, Massachusetts, and London, 1979.) £15.75; $22.50.

DESPITE the generality of its title this book addresses rather specifically the question of magnetohydrodynamic stability as it relates to the currently most promising approach to magnetic confinement of fusion plasmas, the Tokamak. This is perhaps surprising in a book the content of which is primarily didactic. However, the result is a relatively compact book which maintains coherence and covers much ground that is of fundamental importance to the subject.

Dr Bateman begins from a virtually axiomatic statement of the governing MHD equations, moderated by a brief enumeration of effects neglected by the MHD formulation. He then proceeds by way of an illustrative chapter on the Rayleigh-Taylor Instability to a development of the theory of equilibrium and linear stability and a derivation of the energy principle. Cylindrical and toroidal geometries are treated and then high beta questions. Non-linear evolution and resistive instabilities each have a chapter. All this theory is sandwiched between two experimental chapters: the first serving as an introduction and motivation, the last as a comparison between theory and experiment.

Although the mathematical details are included in several cases, the emphasis throughout is on understanding the physical concepts. It is refreshing to read so clear an exposition of the physics in a subject which is so often obscured by a tangle of algebra or mystified by the ubiquitous computer 'simulation'. Not that these are absent, but they are treated with a light, often qualitative, touch, drawing frequently on references to the literature. This approach may feel unsatisfactory for some and it disqualifies the book from being a true reference volume or probably even a self-contained text. However, it greatly enhances its value as an introduction to the subject, and the references (many commendably recent) are there for those who want something heavier to chew on. The provocative questions scattered through the text are great fun.

A serious attempt is made to relate the theory to the experimental context but the connection is not always made really clear. This reflects partly the state of the subject but also an inherent ambivalance in the book between a presentation of fundamentals and a review of current Tokamak MHD research. Given that problem, it seems a pity that Dr Bateman did not choose a rather broader spectrum of applications, to include at least other related toroidal plasma confinement schemes, such as the reversed field pinch in which MHD instabilities are of at least as great importance as in Tokamaks.

MHD Instabilities serves as an excellent introduction for students and researchers in magnetic confinement MHD theory. Its treatment of Tokamak-related problems should prove of great value to the growing numbers in Tokamak research, many of whom are not directly involved in MHD research as such but for whom MHD stability is vital in determining the plasma environment.

I.H. Hutchinson

I.H. Hutchinson is Principal Research Scientist at the Plasma Fusion Center/Frances Bitter National Magnet Laboratory, Massachusetts Institute of Technology, Cambridge, Massachusetts.

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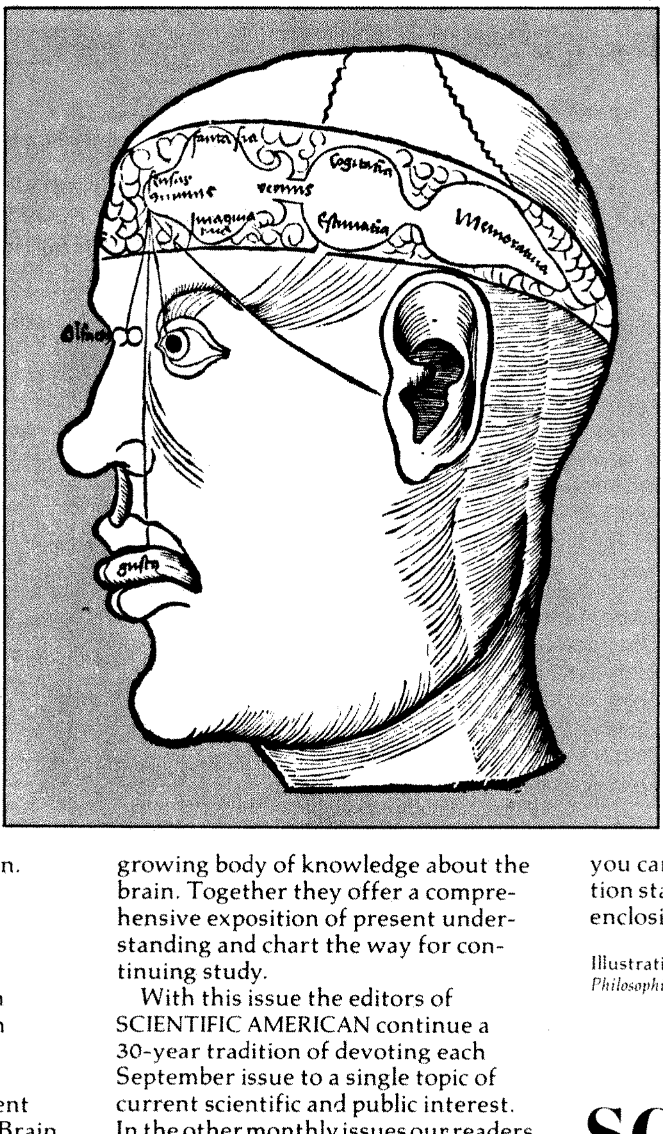

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obituary

Michael Abercrombie

MR MICHAEL ABERCROMBIE FRS, Director of the Strangeways Research Laboratory, Cambridge, who was distinguished for his contributions to the study of cell locomotory behaviour and malignant invasion in tissue culture, died of cancer on 28 May 1979 after a protracted illness. The importance of his scientific achievement lies as much in his almost single-handed establishment of new general aproaches to the problems of development and malignancy as in the details of his own work. His good-humoured courtesy, fairness and deep humanity captured the devotion of all he met throughout the world.

Michael Abercrombie was born on 14 August 1912, at Rhyton Dymock, Gloucestershire. His father, Lascelles Abercrombie, was a distinguished poet and literary critic who later became the Goldsmith Reader at Oxford. In 1931 Michael Abercrombie obtained a Hastings scholarship to read zoology at Queen's College, Oxford. One of his tutors there was Gavin de Beer, an immensely erudite descriptive embryologist, who stimulated his interest in experimental embryology. He left Oxford in 1934 with a 1st in zoology and moved to the Strangeways Laboratory in Cambridge to work with C.H. Waddington who had a dazzling and fascinating influence on him. Here he experimented on the primitive streak in early blastoderms and, for this work, he obtained an Oxford BSc which was then a research degree.

Shortly before the war, he met M.L. Johnson (Jane) at Birmingham University and they were soon married. They spent the war years working together on the degeneration and regeneration of peripheral nerve, a programme of work supervised by J.Z. Young and chosen because of its possible usefulness to wartime surgery. This work was largely carried out in the Anatomy Department at Birmingham but the laboratory was not equipped and Michael had to surrender his superannuation in order to raise money for apparatus. I believe that at the same time he sold his wife's car; her sacrifice surpasses that of Faraday's wife whose linen gown was shredded into strips in order to insulate a coil!

After the war, he took up a lectureship in the Anatomy Department at University College London where J.Z. Young held the chair and here he became reader in charge of the Sub-department of Embryology and eventually, in 1959, a second chair was created for him within the department. Under his leadership, the sub-department soon became a major centre for embryology in Britain and out of it came the *Journal of Embryology and Experimental Morphology*. In 1962 he became Jodrell Professor of Zoology at University College and, in 1970, he succeeded Honor Fell as Director of the Strangeways Laboratory from which he was to retire in the autumn of 1979.

Michael Abercrombie was a founder member of the London Embryologists' Club which first met in November 1947 and which later became the British Society for Development Biology. He was also elected a Fellow of the Institut International d'Embryologie in 1956, a Fellow of the Royal Society in 1958 and was awarded an honorary doctorate by the University of Uppsala in Sweden in 1977.

The main theme of Abercrombie's research was a study of how signals which control locomotory behaviour are communicated between cells. He early noticed that the Schwann cells associated with regenerating peripheral nerve stumps in culture cease to move once the stumps have met and he soon performed the classic experiment of using time lapse cinematography to observe the behaviour of locomoting fibroblasts in a culture consisting of two confronted explants of chick heart tissue. In 1950 he was joined in this work by Joan Heaysman and they started the publication of their results in 1953.

These papers were of enormous importance to biology. They released a powerful new technique — the careful quantitative analysis of cell behaviour — and simultaneously revealed what has since proved to be probably the most important of the behavioural reactions, contact inhibition of locomotion. With Jack Ambrose, Abercrombie made a cytological study of contact inhibition in both normal and malignant cells and the resulting paper became the ninth most highly quoted in cancer in the period 1960-1975. It initiated an interest in the relation of the cell surface to malignancy which swept the cancer research world.

Until his retirement, he continued to work on models of malignant invasion in culture with Joan Heaysman and Pat Stephenson but, in the mid sixties, the emphasis of his research changed to the machinery of cell locomotion and Sue Pegrum, an electron microscopist, joined the group at University College. Later, when I moved with him to the Strangeways Laboratory, this subject became the main interest of our small research group. Among the topics more peripheral to the central theme, he continued his early primitive streak experiments with Ruth Bellairs, worked on liver regeneration with R.D. Harkness and investigated collagen formation during wound healing with D.W. James.

Michael Abercrombie's scientific ability was impressive. His command of techniques and comprehensive knowledge of the literature were matched only by his imaginative insight, his dogged pursuit of a problem and his high standard of objective evidence. His reluctance to speculate or to place too simple an interpretation on his results often made his papers hard going for the reader and sometimes led to the oversimplification or outright misrepresentation of his work by others.

In scientific discussion, as always, he was just and courteous. He spoke little (in his own opinion he was almost neurotically shy) and preferred to listen, but what he did say was very carefully thought out and well worth close study. He was extraordinarily knowledgeable not only within science (he read a minimum of 50 papers in preparation for each lecture to his students) but also in music, painting, literature and politics, rivalling, although he would never have agreed, the breadth of learning which he so admired in his friends Lancelot Hogben, J.B.S. Haldane and Gavin de Beer. Nevertheless, he remained totally modest and unassuming, never averse to humble tasks, even after attaining international distinction. He was patiently tolerant of shortcomings in others and was acutely sympathetic towards oppressed or unfortunate people, spending much of his time unobtrusively helping them.

These, then, were the qualities which have endeared Michael Abercrombie to so many. Perhaps his most extraordinary achievement is that, without exception, all who knew him well had a profound respect and fond regard for him. He overcame the hardships and endured the irony of his final illness with typical stoicism and good humour.

Graham A. Dunn

Julia Bell

JULIA BELL, FRCP died on 26 April 1979, shortly after her 100th birthday. She was one of the pioneers of human genetics and worked at University College London throughout her life.

Like many human geneticists she came into the subject from mathematics, which she had studied at Girton, and she worked as Karl Pearson's statistical assistant from

1908 to 1914. At about that time K.P. became increasingly aware of the lack of medical expertise in the Galton Laboratory, and it was at his suggestion that Julia Bell qualified as a doctor. Applied statistics and medicine formed the basis of the work for which she is famous, the collections of human pedigrees published under the general title of the *Treasury of Human Inheritance*. This was the first systematic attempt to document the distribution of human disease within families, and so anxious was Karl Pearson that these pedigrees should be most scrupulously recorded that in his 1912 preface he promised that the 'Treasury' would contain 'no reference to theoretical opinions.' In Julia Bell he found a fellow perfectionist with regard to the data, but by the 1920s a few mild opinions were obviously more acceptable. Subsequently Dr Bell wrote analytical articles which are among the classic papers of the subject. Perhaps the most notable are her 1940 paper on consanguinity rates and her 1937 paper with J.B.S. Haldane on the linkage between the genes for colour-blindness and haemophilia. This was the first demonstration 'that the principles of linkage which have been worked out for other animals also hold good for man.'

Dr Bell was on the staff of the Galton Laboratory until 1965 when she officially retired, at the age of 86! Although never Galton Professor of Human genetics herself she outlived the first three, Pearson, Fisher and Penrose, and in her 90s was still sufficiently concerned with the Laboratory to remonstrate with the Galton Professor when she felt it to be necessary! She was a supporter of the Women's Suffrage Movement, but never a militant. She was something rarer, one of the first generation of women to engage in professional scientific research, and to do it supremely well.

Elizabeth B. Robson

N.D. Riley

NORMAN DENBIGH RILEY, CBE, who died on 26 May 1979 at the age of 88 was an eminent British entomologist with a high reputation as a lepidopterist and as a tireless and effective worker in the interests of international entomology.

For nearly a quarter of a century he was head (Keeper) of the Department of Entomology in the British Museum (Natural History), and between the 1930s and 1950s saw it transformed from a dusty insect storehouse into a modern research institution. We may claim for him, as such a modest man would never claim for himself, that he was largely responsible for the high regard in which the Department of Entomology is still undoubtedly held internationally.

Fifty years ago service to one's country was more esteemed than it is today, and in 1932 when E.E. Austen (a military man himself) retired as Keeper of Entomology he was instrumental in securing Riley's appointment as his successor in preference to F.W. Edwards. Riley had served in the army with distinction for the whole length of the Great War, rising to the rank of Captain, whereas Edwards — the scientifically more qualified man — had been a non-combatant pacifist through his Quaker convictions.

While Riley might have been fortunate at this juncture in his life, his promotion to Keeper was to prove amply justified. He had a taste for administration unusual in entomologists and soon became the architect both figuratively and almost literally (as he did much to plan the present departmental building) of an expanding Department of Entomology. During his Keepership the insect collections grew enormously, often through purchase of specialized collections negotiated by Riley and there were comparable increases in the department staff. Riley himself was a gregarious man, and when his staff became large enough he encouraged more contact amongst them by organizing annual staff dinner dances, which proved a popular innovation in an otherwise rather stuffy museum. During the Second World War he organised the evacuation of large parts of the collection and the library to safe quarters, and their return after the war, although he remained in South Kensington. The CBE was conferred on Riley in 1952.

In his Presidential Address to the Royal Entomological Society, Riley amused his audience by recalling when his small daughter was overhead to say "My daddy doesn't work — he goes to the Museum." In this she had caught Riley's character to a nicety, for to him work and pleasure were synonymous, and long after his official retirement in 1955, until his death in fact, he was a regular visitor to his beloved collection of butterflies in the Museum.

Butterflies were Riley's life-long scientific interest, and one that he was able to indulge professionally from the time of his appointment to the Museum staff as an Assistant in 1911 (which followed on the heels of a short period learning entomology while a demonstrator at the nearby Imperial College). It was not until his return from the war in 1919, however, that Riley published his first modest paper, describing some new Brazilian species of butterflies, but from then on he published regularly for over half a century, his bibliography of about 400 works including books, editorials and reviews as well as scientific papers. His scientific contributions to lepidopterology will be assessed in specialist journals, but mention should be made of his book written in collaboration with his friend Lionel Higgins entitled *A Field Guide to the Butterflies of Britain and Europe*: although only published in 1970 this has already run through several editions and has been issued in nine languages.

Riley lived as a boy in South London, where he was born in the suburb of Tooting on 20 September 1890 and where he was educated at Harlington School, Balham and Dulwich College. A neighbour was Richard South, doyen amongst British Lepidopterists, and it was he who stimulated Riley's childhood interest in butterflies — so effectively as it turned out that Riley even on the eve of his death was discussing butterfly nomenclature with a friend.

Butterflies in spite of, or perhaps because of, their popularity have always had their problems with scientific names, and Riley was inexorably drawn into the intricacies of nomenclature, with the result that he served for 15 years (from 1950 to 1965) on the International Commission for Zoological Nomenclature. As commision Secretary and member of its editorial Committee he was the mastermind behind the current edition of the International Code of Zoological Nomenclature and bore much of the burden of its production.

He was prominent also in international biology through his election to the International Entomological Congresses and his long connection with Congress organization. For a time Riley served as Chairman of the Entomological Section of the International Union of Biological Sciences. Responsibilities of this kind involved him in occasional distant travel, but most of his collecting was done in Europe. At home Riley's energies were poured into Society affairs. He was a Fellow of the Royal Entomological Society for 67 years (elected in 1912). For the last twenty of these, until his death he held the rare distinction of a Life Fellowship. Riley served on the Society's council (1921-23, 1929-30), and as its Secretary (1926-29, 1941-51), Treasurer (1939-40) and President (1951-52).

He belonged to the British Entomological and Natural History Society for an even longer period, having joined as a boy in 1902, and was its President in 1923-24. This Society gave him special links with many amateur specialists who were keen readers of *The Entomologist* a monthly journal largely devoted to Lepidoptera that Riley owned and edited for thirty-six years (1923-1959). A position he relished was that of organizer over many years of the Verrall Supper, an annual gathering of professional and amateur entomologists; in a long life he missed only two of these occasions and was present at the very first in 1912.

Norman Riley enjoyed every moment of a hard-working life and was the perfect examplar of those qualities that in a more gentlemanly age were laid down for the conduct of British Museum staff — that they should be "persons of honour, integrity and liberality." He is survived by his widow, a son and daughter.

Pamela Gilbert

Georgiana M. Bonser

DR GEORGINA M. BONSER (née Duthie), MD (Manchester), FRCP (London), a leading woman doctor engaged in cancer research in England, died on 9 June 1979 at the age of 81.

She was born on 5 May 1898 and studied medicine in Manchester University and at King's College Hospital, London, qualifying in 1920. By 1923, she had been awarded her MD with distinction and won a travelling fellowship which enabled her to spend a year at the Institut Pasteur in Paris. While there, she met her future husband, Kenneth Bonser, an architect. In 1927 she began her long association with Leeds University and its hospitals. Fortuitously, her interests were channelled into cancer research by her chief, Professor Matthew Stewart. She was a woman with a strong sense of purpose who saw two challenges in her life, the problems of cancer and the need for women in the medical profession to establish their rightful place in an essentially male preserve. Dr Bonser's efforts were eventually to be recognised by her election to the chairmanship of the Leeds branch of the British Medical Association, in 1953, and presidency of the Medical Women's Federation in 1959, which speak for her tenacity and political acumen.

But to appreciate her real achievement, it must be recalled that cancer research, when she began, paid the handsome salary of £200 a year and it was necessary for her to take a part-time job in pathology in order to have enough money to live on. She held several posts in the Leeds hospitals and university until she finally became reader in experimental pathology and cancer research and a consultant pathologist at St. James's Hospital and lived to see this hospital translated from a poor law institution to a modern teaching hospital.

Dr Bonser's contribution to knowledge has been in the studies of chemically induced carcinogenesis of the bladder. Although the association of bladder cancer and the dyeing industry had been established in the late nineteenth century, all efforts to induce bladder tumours with the products from the dyeworks or urine of workers affected with bladder cancer were a failure. Dr Bonser, in 1937, reviewed these disappointing results which were somewhat reminiscent of the research for some of the more elusive infective agents some 50 years previously. Hueper's report that the feeding of large doses of ß-naphthylamine to dogs produced bladder cancer was the turning point. By 1943, Dr Bonser reported that the following feeding purified ß-naphthylamine to dogs for 5 years, bladder cancer could be induced. This helped to remove suspicion from α-naphthylamine and identify one hazard in the dyeing industry.

She soon realised that the dog was too cumbersome and expensive an animal for research and concentrated on developing models in small animals. From this time, progress was much more rapid as the waiting time to see the result of an experiment was then a matter of one to two years. Dr Bonser's team confirmed that 2-acetylaminofluorene when fed to mice was a bladder carcinogen; this was the first in a long series of experiments that resulted in her being an expert on the interpretation of the pathology of the bladder in laboratory animals.

It became clear to Dr Bonser that she needed specialist help; in part it came from Professor L.N. Pyrah, a urological surgeon who helped with surgical techniques in the animals but the whole programme really became most effective when she recruited D.B. Clayson, a young chemist and J.W. Jull, a biologist to help her. There then followed a long haul in which the metabolism of several potential bladder carcinogens was studied and the carcinogenic properties of their various derivatives tested in her systems. An important part of the techniques developed by Dr Bonser's team was the direct implantation of carcinogens in a variety of different vehicles into the bladders of rats and mice. The net result of their efforts over the next 15 years was to put one part of the bladder carcinogenesis story on to a sound scientific basis. Its eventual impact reached far beyond the dyeworks to embrace the workers in rubber and cable making industries who came into contact with ß-naphthylamine. Dr Bonser's strength lay in a sense of purpose, her strong discipline and sound knowledge of general pathology as well as her natural clinical practice; in 1961 she was co-author of a book on the pathology of breast cancer.

In the University her research was solidly based on her nose for a good experiment and skill at being able to interprete the results. Many others who have delved in this field had been hopelessly wrong as they could not tell the difference between hyperplasia of the bladder mucosa from that of early carcinoma. One of her last tasks after her retirement was the writing with one of her former pupils, of a chapter on the pathology of the bladder in experimental animals in the handbook produced by the International Agency for Cancer Research.

Today, with the flood gates open to pour money into testing for carcinogens, it is salutary to reflect that in the smoke and grime of pre-war Leeds, this small, determined and formidable woman was quietly laying the corner stones of the work. The combination of her keen mind and dogged persistence was rewarded by achieving a few lasting goals, but what is most important, she was one of those pioneering women scientists ahead of her time. Georgiana Bonser had been a great credit to her University and dealt a nasty jolt to male chauvinism in medicine, both of which must have given her the satisfaction of a life well spent.

Edward H. Cooper

Sir Peter Venables

SIR PETER VENABLES died at his home in Birmingham on Sunday 17 June 1979. He was 74. His passing leaves a gap in the world of education in Britain that cannot easily be filled.

Peter Venables started his career as a chemist; to use his own words as "a mere chemist." He emerged from Liverpool University in 1925 with a first-class honours degree in chemistry. It was typical of the man, and of his later career, that, despite the quality of his first degree, he should, before embarking on study for a PhD, have spent a year acquiring an Education Diploma. He then spent four years in research, obtaining a PhD in 1928 followed by 2 years of postdoctoral work.

For the next 11 years his career followed the standard pattern of a teacher in higher education. First as a Lecturer and later as a Senior Lecturer in the Leicester College of Technology (now the Leicester Polytechnic) and then as Head of the Science Department at the South-East Essex Technical College he continued to teach and to carry out research in chemistry. But while at Leicester his lifelong interest in the visual arts was stimulated by the close contacts he made with the staff of the College of Art.

But it was in 1941, at the age of 37, that he took the critical step that was to determine the path of his future career. He accepted the post of Principal of the Municipal College at Southend-on-Sea. This was a move into academic administration. From now on Peter Venables was not "a mere chemist"; he had entered the curious world where education and administration are blended. Academic excellence is a necessary passport to enter this world but in it academic work must take second place (and often third or fourth place), yielding to the need to provide leadership to a company of men and women characterised by independence of mind and often of equal independence of action. He had the qualities required for success, an absolute integrity of purpose which he would pursue, as the occasion required, with consummate patience, with a formidable show of strength or even, where necessary, with guile. He had a mischievous wit and could school his countenance to betray no expression whatsoever as he uttered his most outrageous quips. All in all he inspired in his friends and colleagues both an absolute trust and a deep affection, a combination that inevitably makes a leader of men, and a leader he was to be for the rest of his career.

From Southend-on-Sea he moved in 1947 to become principal of the Royal Technical College at Salford. During the next nine years he laid there the foundation upon which, much later, that college was to become the University of Salford. His qualities were recognised more widely by his election as President and later Chairman of the Council of the Association of Principals of Technical Institutions.

In 1956 he moved again, this time to the College of Technology in Birmingham. Here for a decade he presided over its development as the first College of Advanced Technology in the country, of its transition from a CAT to a technological university and, for a further three years as the first Vice-Chancellor of the University of Aston. He was, therefore, deeply involved in the pedagogical and political arguments about the growth of university and technological education throughout a period of almost revolutionary change. He brought to them his wisdom, his vision and his devotion to a humanitarian ideal. His recognition of the need to draw together the interests of industry and education led him to pioneer the sandwich course which found an honourable place in the spectrum of educational provision.

He also found time and energy to give unstinting service as Chairman, President or member of a whole range of other educational institutions. Of particular note, perhaps was the fact that the British Broadcasting Corporation and the Independent Television Authority, if they agreed on nothing else, both chose him to act over the same period as Chairman of their Further Education Advisory Council and Adult Education Advisory Committee respectively — surely a unique distinction in educational broadcasting.

In 1968 he was also Vice-Chairman of the Committee of Vice-Chancellors and Principals and it was while he held this post that he accepted an invitation from Jennie Lee to become Chairman of the Planning Committee of the Open University. It was an inspired choice; but it came as a surprise and a shock to many of his colleagues who did not share his faith in this new educational venture. But it was a venture that called for all the qualities that he had in abundance, humanitarianism, egalitarianism, the desire to harness technology to the service of society. He imprinted these qualities upon the new institution not only during the planning phase but also, as its first Pro-Chancellor and Chairman of Council, during its early formative years. Throughout that period and, indeed, right up to his death, he remained the consultant architect; his was the grand design even though others might build particular bits.

Numerous honours came his way but he remained just the same, a straightforward and uncomplicated chap. For 47 years he was sustained by a very happy and stable home. His wife, Ethel, a distinguished scholar in her own right, shared his hopes and his motives. Our sympathy goes to her and to the children in their sad loss, a loss that seems as personal to many of us as it must be to them.

Walter Perry

H.B.D. Kettlewell

HENRY BERNARD DAVIS KETTLEWELL died on 10 May 1979, aged 72. He was one of those dedicated scientists who gave up work as a medical practitioner in order to conduct biological research, so that his professional life falls into two parts.

He was the son of Henry Kettlewell and Kate Davis. In 1936 he married Hazel Margaret, daughter of Sir Frank Wiltshire, having one son and one daughter.

He was educated at Charterhouse and Caius College, Cambridge. He obtained the degrees of MA, MB, and B Chir, and acquiring the status of MRCS and LRCP, he held several hospital appointments. During the war of 1939-45 he worked at the Woking War Hospital, subsequently taking up general practice at Cranleigh, Surrey.

In 1949 he emigrated to South Africa, researching for a time on locust control. He also conducted expeditions to the Kalahari, Belgian Congo and Mozambique. Characteristically, he drove his motor car from Cape Town to Alexandria when in 1952 he decided to take up a Nuffield Research Fellowship in genetics in the Department of Zoology at Oxford. Two years later he became a Senior Research Officer, a position he held until his retirement in 1974. He was elected a Fellow of Wolfson College, Oxford and obtained the degree of DSc at Oxford in 1975.

During his research career, he undertook lecture tours in the USA and Canada and visited Brazil for *Life Magazine* on the occasion of the centenary of the *Origin of Species*. He was co-founder of the Rothschild-Cockayne-Kettlewell Collection of Lepidoptera now housed in the Natural History Museum, South Kensington. This is of a unique type, illustrating the genetics of butterflies and moths with full references to the literature accompanying each set of specimens.

Bernard Kettlewell was the finest living ecologist of the Lepidoptera. Moreover, he was blessed with the good luck which ought to attend devoted field workers. On one occasion he found himself surrounded by large numbers of the Bath White butterfly *Pontia daplidice* in South Cornwall and had a specimen of this and of another fabulously rare species the Short tailed Blue *Everes argiades*, in his net at the same time.

It is, in particular, with the study of industrial melanism in the Lepidoptera that Kettlewell's name will always be associated. That phenomenon involves the most spectacular, though not the most profound, evolutionary change which has ever been witnessed. He showed that over a hundred species are affected by it in Britain alone. The correct interpretation of the subject had been handicapped by the mistakes and failures of others in the past. These included the wholly incorrect explanation, depending on mutation-pressure, put forward by Heslop-Harrison. Moreover, both ornithologists and entomologists had entirely failed to realise that birds hunting by sight may selectively destroy resting moths. It was the triumph of Kettlewell to show by much detailed observation and experiment that the explanation of this occurrence by mutation-pressure is invalid, and to confute those who maintained it by collaborating with N. Tinbergen to produce brilliant cinematograph pictures demonstrating such predation.

Kettlewell was able to analyse the whole subject of industrial melanism with great success, using in particular the Peppered Moth, *Biston betularia*, of which he bred thousands of specimens for study in the field. Moreover, by various recapture methods he obtained definite information on their survival. Such melanism is confined to species that rest exposed, matching their background. Kettlewell demonstrated that the black form survives better in polluted areas but is eliminated in normal countryside. He also studied the genetics of the situation and showed that the melanism is controlled as a polymorphism, with heterozygous advantage.

This work has important ancillary applications, including an impressive demonstration of the evolution of dominance obtained by crossing the British Peppered Moth with a related Canadian species. Also the evolution of a super-gene in the Oak Eggar, *Lasiocampa quercus*. His work on the Lepidoptera extended in many other directions, including the detection of speciation in its early stages due to isolation by distance. He also carried out a remarkable analysis of variation in the Scarlet Tiger Moth, *Panaxia dominula*, conducted partly in the garden of his country house between Oxford and Banbury.

He recorded his research results in many articles. He also wrote several books, the most important being *The Evolution of Melanism*, 1973 (Clarendon Press, Oxford).

His work gained considerable recognition. He was awarded the Darwin Medal of the USSR in 1959, and the Mendel Medal in Czechoslovakia in 1965. Throughout his research career Kettlewell was constantly demonstrating the importance of studying evolution by observation and experiment using the techniques of ecological genetics.

E.B. Ford

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APPOINTMENTS VACANT

PRE AND POSTDOCTORAL POSITIONS

are available for individuals with microbiology background for studies on the protein synthesising system with special emphasis on ribosomes — topography, biogenesis and function. Teaching opportunities are available. Reply with résumé to: Dr Bruce H. Sells, Professor and Director, Laboratories of Molecular Biology, Faculty of Medicine, Health Sciences Centre, Memorial University of Newfoundland, St John's, Newfoundland A1B 3V6, Canada. W350(A)

THE UNIVERSITY OF PAPUA NEW GUINEA

(Port Moresby)

Applications are invited for the post of

PROFESSOR OF HUMAN BIOLOGY

within the
FACULTY OF MEDICINE.

Applicants should have a distinguished academic record with either Gross Anatomy, Histology, Biochemistry or Physiology as their field of interest. A medical qualification would be a distinct advantage, although a person who is not medically qualified may apply. Teaching and/or research experience in a developing country would also be an advantage. The appointee will provide academic leadership, participate in undergraduate and graduate teaching in the basic medical sciences, and stimulate research. He/she will also encourage the development of Papua New Guinea national academic staff. The Professor will not necessarily have any administrative responsibilities although he/she can be elected to positions carrying such responsibilities. The Department of Human Biology is responsible for teaching the basic medical sciences to undergraduate and postgraduate students. Courses offered include Gross Anatomy, Histology, Embryology and Neuroanatomy; Biochemistry, Nutrition and Genetics; Physiology and Neurophysiology, and Pharmacology.

Salary: K18,675 pa (£1 Sterling = K1.57) pa. (In addition a clinical allowance of K2,000 pa will be payable). Family passages; baggage allowance; 3 years contract; 24% gratuity of salary of which 5% is taxable; support for approved research; rent-free accommodation; annual leave; generous education allowances; medical assurance scheme.

Detailed applications (2 copies) with curriculum vitae and naming 3 referees to be sent direct to the Secretary, Box 4820, University PO., Papua New Guinea by 8 November, 1979. Applicants resident in the UK should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 1DT. Further details may be obtained from either address. 3203(A)

TECHNICIAN or JUNIOR TECHNICIAN required by the Institute of Cancer Research in association with the Royal Marsden Hospital at Sutton, Surrey. Candidates should have an interest in cell culture and, ideally, some previous experience in this field and also in enzymology. The individual appointed will take part in a new MRC Project on clinical and laboratory aspects of head and neck cancer, commencing September — October, 1979. Salary in scale £3,261 to £3,813 p.a. (Technician) or £2,169 to £3,015 p.a. (Junior Technician), plus London Allowance of £354 p.a. (scales under review). Salary will depend on age and qualifications, two 'A' levels in science subjects for Junior Technician and either a Degree, HNC or equivalent qualification for a Technician appointment. Applications in duplicate with the names of two referees to the Secretary, Institute of Cancer Research, 34 Sumner Place, London, SW7 3NU, quoting ref. 301/B/73. Closing date for applications, 28th September, 1979. 3149(A)

MEDICAL RESEARCH COUNCIL OF NEW ZEALAND

DIRECTOR

VIRUS RESEARCH UNIT

Applications are invited from medically and non-medically qualified virologists with proven experience in research for the position of Director of the Council's Virus Research Unit which is located in the Microbiology Department of the University of Otago, Dunedin, New Zealand. Applications from clinicians and research workers interested in developing a programme in association with hospital-based diagnostic virology would also be entertained.

The salary will be based on qualifications and experience but will be equivalent to that of Associate Professor in the University. In the case of an exceptionally well-qualified applicant Council will consider an appointment at a level equivalent to that of Professor.

Salary: Associate Professor (medical) NZ$27,365-30,860; Associate Professor (non-medical) NZ$21,565; Professor (medical) NZ$30,365-33,865; Professor (non-medical) NZ$23,865-29,865.

It should also be noted that wage orders increasing these salaries by approximately 15% are expected to be applied in the near future.

Further particulars are available from the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF; from the Registrar of the University of Otago, PO Box 56, Dunedin, New Zealand, or direct from J.A. Borrows, Technical Secretary, P.O. Box 6063, Dunedin, New Zealand.

Applications close in New Zealand and London on 1 December, 1979.

3177(A)

DEPARTMENT OF BIOPHYSICS

KING'S COLLEGE

London

and

NATIONAL INSTITUTE FOR MEDICAL RESEARCH

Applications are invited for a postdoctoral position, tenable for one year in the first instance, with the possibility of renewal for three years, supported by W.H.O. The research concerns the mechanism by which the malaria parasite enters the host red cell. Experience in biochemical or biophysical aspects of membrane and cytoskeleton research would be an advantage; prior knowledge of parasitology is not essential.

Applications with curriculum vitae and names of two referees to D.M. Drummie, Administrative Assistant, Department of Biophysics, King's College, 26-29 Drury Lane, London WC2. 3163(A)

EAST MALLING RESEARCH STATION

LIBRARIAN

Librarian to take responsibility for library and associated services at major horticultural research station conducting a wide range of fundamental and applied studies on problems of commercial fruit growing.

Candidates must have degree in biology, horticulture or agriculture with postgraduate librarianship or information science qualification with at least 5 years professional experience.

'On-line' (Lockheed) experience an advantage.

Salary in the scale £5,520 to £6,519 (£5,950 to £7,250 from 1st January 1980); non-contributory pension scheme.

Application form and further details from Secretary, East Malling Research Station, Maidstone, Kent. 3146(A)

BBC OPEN UNIVERSITY PRODUCTION CENTRE

Vacancies exist for production staff at the Production Assistant level.

Candidates must have a good Honours degree in one of the following: Geology, Biochemistry, Mathematics, Psychology (or another Social Sciences subject). At least two years post-university experience and the ability to work on a wide range of subject disciplines is essential.

Successful applicants, who will be given production training if necessary, will work on all aspects of the production of radio and television programmes for the Open University.

Open University Production Centre is based at Alexandra Palace but will move to Milton Keynes in 1981.

Salary: £6,385 to £8,405 p.a. Plus unconsolidated allowance £617 p.a. Relocation expenses considered.

Telephone or write immediately for an application form, enclosing addressed envelope and quotiong reference 2527/N to: *Appointments Department, BBC, London W1A 1AA. Telephone 01-580 4468 Ext. 4619.*

3193(A)

BBC tv

A.R.C. ANIMAL BREEDING RESEARCH ORGANISATION

IMMUNOGENETICISTS

SENIOR SCIENTIFIC OFFICER/SCIENTIFIC OFFICER

Applications are invited for two posts:

Project: To study histocompatibility antigens in tropical cattle and their possible association with disease resistance. Part of the project, involving Theileriosis, will be in collaboration with the Centre for Tropical Veterinary Medicine (Edinburgh).

Duration: Three years.

Location: The posts will be based in the Department of Immunology, ABRO, Edinburgh, where the appointees are expected to spend one year, with the remaining two years in Africa.

Qualifications: (1) SENIOR SCIENTIFIC OFFICER (Project Leader):
1st or Upper 2nd Class Honours in a biological science, with at least 4 years relevant post-graduate experience preferably including immunology or genetics.
(2) SCIENTIFIC OFFICER
A degree or HND in a biological science with, preferably, immunological or genetical experience.

Salary: (1) SSO: within scale £6,332 to £8,705
(from 1.1.80) (2) SO: within scale £3,591 to £5,486

Starting salary according to qualifications and experience.

Each post will attract tax free overseas allowances and terms similar to those for Ministry of Overseas Development Technical Assistance appointments and is superannuated in a non-contributory scheme.

Applications: Full curriculum vitae with names of two referees to the Secretary, ARC Animal Breeding Research Organisation, West Mains Road, Edinburgh EH9 3JQ, by 13th October, 1979 from whom further particulars are available.

3161(A)

UNIVERSITY OF DUBLIN
Trinity College

LECTURESHIPS IN PHARMACOLOGY
(2 posts)

Applications are invited for two Lectureships in the Department of Pharmacology, Trinity College, Dublin, which are available immediately.

In the case of the first position, preference may be given to medically qualified applicants; for the second position, preference will be given to candidates with qualifications and experience in Pharmacology, Pharmacy or a related area.

Salary scale: £5,078 to £10,038 p.a.

Appointment will be made at a point appropriate to the qualifications and experience of the successful candidates; in the case of the first position above, appointment may be made within the full range of the Lecturer scale; in the case of the second post, appointment is likely to be made within the range £5,078 to £6,462 p.a. There is a non-contributory pension scheme.

Application forms and further particulars relating to these posts may be obtained from:

The Establishment Officer
West Theatre
Trinity College
Dublin 2

who will receive completed applications **up to Friday, 28th September, 1979.** 3192(A)

UNIVERSITY OF OXFORD
in ASSOCIATION with ST. HUGH'S COLLEGE
LECTURERSHIP IN ASTRONOMY

Applications are invited, from men and women candidates, for a University Lecturership in Astronomy, in the Department of Astrophysics, to be filled from 1 January 1980 or as soon as possible thereafter.

Preference will be given to applicants with a broad knowledge of astrophysics whose research interests lie in the field of optical astronomy (including the ultraviolet and infrared regions).

The stipend will be according to age on the scale £4,232 to £9,000 (under review) with superannuation.

The lecturership may be held with a tutorial fellowship in physics at St. Hugh's College and a linked lecturership at St. Edmund Hall. Applicants should state whether they wish to be considered for the college posts.

Further details of the university and college posts may be obtained from Professor D.E. Blackwell, Department of Astrophysics, South Parks Road, Oxford, OX1 3RQ, to whom applications (one typed copy) should be sent before 15 October, 1979. Applications should contain a statement of qualifications, a list of publications, an outline of a proposed research programme and the names of three referees. 3155(A)

GREEK BIOCHEMISTS
MOLECULAR BIOLOGISTS

The Laboratory of Biological Chemistry, University of Ioannina Medical School, invites applications for two positions of

ΕΠΙΜΕΛΗΤΑΙ

starting December 1st, 1979 or later. Applicants must be fluent in Greek, and have a PhD in biochemistry/molecular biology, or an MD with strong interests and experience in the above subjects.

The successful candidates will be expected to take part in the teaching program for about 100 medical students and in the research interests of the laboratory. These interests currently are in enzymology, protein chemistry, and membranes. In addition, a Center for Brucellosis, an endemic disease in the area, is under consideration. The basic salary is about 19,000 Drs per month before taxes and is expected to be supplemented from other sources.

Applicants should forward their curriculum vitae with bibliography, two or more letters of recommendation, and a brief outline of research interests to: Orestes Tsolas, PhD, Professor, Laboratory of Biological Chemistry, University of Ioannina Medical School, Ioannina, Greece. (Telephone 0651-33442). W351(A)

THE UNIVERSITY OF MANCHESTER. Research Technician (grade 5) required to work in the Department of Anatomy. Applications are invited for the post of research technician to work in the area of experimental neurology. Applicants must hold HNC or equivalent and have at least 8 years' laboratory experience. Competence in a wide range of modern techniques in histology and microscopy including autoradiography is essential. Previous experience in research work is required and a working knowledge of the use of neuroanatomical, neurophysiological or neuropharmacological research techniques would be a considerable advantage. Salary on the interim scale £3,700 to £4,320 p.a. together with further comparability payments and subject to overall review as from 1st October 1979. Applications giving details of age, qualifications, experience and the names of two referees should be sent to Professor P.F. Harris, Department of Anatomy, Medical School, University of Manchester, Manchester M13 9PT, by 30th September 1979. 3109(A)

LINCOLN COLLEGE
New Zealand
LECTURER IN ANIMAL NUTRITION

The Council of Lincoln College, University College of Agriculture, invites applications for appointment to the position of Lecturer in Animal Nutrition. Applicants must hold a degree in Agricultural Science, Veterinary Science or some related branch of science, with a higher degree in animal nutrition. Preference will be given to those candidates with experience of research in the physiology of digestion and/or control of feed intake in ruminants. The appointee will be attached to the Animal Science department within the Animal Sciences Group.

The salary scale for lecturers ranges from NZ$11,894 to NZ$14,615. It is expected that the recent Ruling Rate Survey increase of 10.4% and General Wage Order of 4.5% will be applied.

Travel and removal expenses will be reimbursed up to specified limits. New Zealand Government Superannuation will be available.

Conditions of Appointment giving further details of this position can be obtained from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or from the Registrar of the College.

Applications close on 31 October, 1979. 3167(A)

THE MIDDLESEX HOSPITAL MEDICAL SCHOOL
(University of London)
SCHOOL OF PATHOLOGY
POST-DOCTORAL BIOCHEMIST RESEARCH ASSISTANT

Applications are invited from post-doctoral biochemists to collaborate in a 3-year project, supported by NCI (USA) funds, to study carcinogenesis *in vitro* in human bladder explants.

Applicants should have worked in the field of carcinogen metabolism and have appropriate experience in techniques for studying the adducts formed between carcinogens and DNA or proteins. Technical assistance will be available.

The appointment is for one year in the first instance, renewable for a further two years. Starting salary on the scale effective 1 October 1979 within the range £4,835 to £6,858 inclusive of London allowance according to age, qualifications and experience.

Please send applications, with curriculum vitae and the names and addresses of two referees to Dr R.M. Hicks, School of Pathology, The Middlesex Hospital Medical School, London W1P 7LD by 1 October 1979. 3144(A)

THE UNIVERSITY OF MANCHESTER
BARKER CHAIR OF CRYPTOGAMIC BOTANY

Applications are invited for the Barker Chair of Cryptogamic Botany which will fall vacant on October 1st, 1980 following the retirement of Professor John Colhoun. The University has a preference for candidates with interests in the applied fields. The other Chair in Botany is the George Harrison Chair to which Dr Elizabeth G Cutter has been elected. Particulars of the appointment may be obtained from the Registrar.

Applications (2 copies, 1 suitable for photocopying), giving full details of qualifications, experience, research etc., and the names and addresses of three persons to whom reference may be made, should be sent to The Registrar, The University, Manchester, M13 9PL by October 10th. 3159(A)

THE MIDDLESEX HOSPITAL MEDICAL SCHOOL
(University of London)
SCHOOL OF PATHOLOGY
MEDICAL LABORATORY SCIENTIFIC OFFICER BIOCHEMIST
(Whitley Council)

Applications are invited from Scientific Officers to assist in a research project involving carcinogenesis *in vitro* in human tissues. Candidates should be associates of the IST, IMLT or equivalent and must have had experience in a biochemical laboratory preferably working on the metabolism of carcinogens.

The appointment is funded by the NCI (USA) and is for one year initially, renewable for a further two. Salary in the range £4,050 to £5,034 depending on age, qualifications and experience.

Please send applications, with curriculum vitae and the names and addresses of two referees to Dr R.M. Hicks, School of Pathology, The Middlesex Hospital Medical School, London, W1P 7LD, by 1 October 1979. 3145(A)

FU BERLIN

INSTITUT FÜR KRISTALLOGRAPHIE

Applications are invited for a

POSTDOCTORAL RESEARCH POSITION IN STRUCTURAL STUDIES ON BIOPOLYMERS

tenable for one/two years with possible prolongation. Salary in scale 40000 to 60000 DM depending on age and experience.

The successful candidate is expected to have sound experience in biochemistry/molecular biology. Background in structural studies using X-ray diffraction and other physical methods is desirable. Work will focus to structure of nucleic acids and/or bacterial cell walls.

Please send applications with curriculum vitae, qualifications and names of two referees to Prof. Dr. Bradaczek, Institut für Kristallographie, Freie Universität Berlin, Takustraße 6, D-1000 Berlin 33. W349(A)

GUY'S HOSPITAL MEDICAL SCHOOL

Unit for Metabolic Medicine

DEPARTMENT OF MEDICINE

GRADUATE OR POSTDOCTORAL RESEARCH ASSISTANT

(Biochemist or Pharmacologist)

required for two years in the Unit for Metabolic Medicine to work on a research project concerned with insulin metabolism and metabolic control in diabetes mellitus.

Salary according to qualifications and experience, with London Allowance.

Apply in writing, with full curriculum vitae, to the Secretary, Guy's Hospital Medical School, London Bridge, SE1 9RT, quoting Ref. MM1. 3180(A)

THE ROWETT RESEARCH INSTITUTE

Bucksburn, Aberdeen, AB2 9SB

PATHOLOGIST/CELL BIOLOGIST

Scientific Officer/Higher Scientific Officer Grade

Applications are invited for a post in the Institute's Department of Experimental Pathology, which studies the pathology of nutritional disease in farm animals, and diseases associated with specific husbandry practices.

The post is for a morphologist who has a particular interest in cytochemical and ultrastructural methods and their application to the study of growth, and nutritional and idiopathic neuromuscular diseases in farm animals.

Candidates should possess a 1st or upper 2nd class Honours degree in the biological sciences. At least two years relevant postgraduate research experience is required for HSO grading.

Salary Scales: Scientific Officer £3,289 to £5,085 p.a. or Higher Scientific Officer £4,727 to £6,211 p.a. with placing according to qualifications and experience. On 1 January 1980 these scales will be increased to: S.O. £3,591 to £5,486 p.a. and H.S.O. £5,097 to £6,737.

There is a non-contributory Superannuation scheme.

Further details of the post may be obtained from the Secretary of the Institute, to whom applications should be submitted by 5 October 1979 together with the names of two people to whom reference can be made. 3204(A)

UNIVERSITY OF LONDON

INSTITUTE OF NEUROLOGY

RESEARCH ASSISTANT

Graduate research assistant, preferably with research experience in physiological psychology or psychopharmacology, required for an MRC-supported project on behavioural effects of antischizophrenic drugs in rats.

Appointment for one year in the first instance. Salary and superannuation on University scale.

Apply with curriculum vitae and names of referees to Dr L.J. Herberg, Experimental Psychology Laboratory, Institute of Neurology, National Hospital, Queen Square, London WC1. 3148(A)

UNIVERSITY OF CAMBRIDGE

DIVISION OF VIROLOGY

DEPARTMENT OF PATHOLOGY

POSTDOCTORAL RESEARCH ASSOCIATE

Applications are invited for a five-year appointment with a team studying the molecular biology and genetics of influenza viruses. Previous post-doctoral experience in biochemistry, genetics or virology desirable. Starting salary up to £5,488 p.a. depending upon age and experience.

Applications with curriculum vitae and the names of two referees should be sent as soon as possible to Dr B.W.J. Mahy, Division of Virology, Department of Pathology, University of Cambridge, Laboratories Block, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ, from whom further details may be obtained.

RESEARCH TECHNICIAN

required as soon as possible to join a team studying the molecular biology of influenza viruses. The post is funded until September 1984. Qualifications: good degree, HNC or equivalent in biochemistry, genetics or micro-biology. Experience in tissue culture or virology advantageous. Starting salary up to £3,226 p.a.

Applications and enquiries to the Superintendent, Department of Pathology, Univeristy of Cambridge, Tennis Court Road, Cambridge CB2 1QP. Telephone 0223-64631, extension 27.

3208(A)

CSIRO AUSTRALIA

Post Doctoral Research Fellow

Centre for Animal Research and Development Bogor Indonesia

FIELD: Poultry Research

CSIRO has a broad charter for research into primary and secondary industry areas. The Organization has approximately 7,000 employees – 2400 of who are research scientists – located in Divisions and Sections throughout Australia.

DUTIES: An experienced poultry research scientist is required to lead a research programme on Chicken Management and Nutrition at the Centre for Animal Research and Development in Indonesia. The aim is to develop appropriate management and feeding systems for both the large and small-scale Indonesian poultry producer. The primary research effort will be directed to improving the efficiency of utilization of indigenous feedstuffs for chicken meat and eggs. The appointee will be expected to work closely with scientists working in an allied Duck Research Programme.

The Centre has excellent facilities and staff for nutritional studies with chickens and there is a modern analytical laboratory and veterinary section to provide back-up service. Existing studies have focused on the evaluation of local feedstuffs and development of new feed resources. The new appointee will be expected to further the research in these areas, and to consider the general problems of management of small chicken flocks at the village level.

QUALIFICATIONS: PhD and wide experience in the field of poultry research with an understanding of poultry nutritional experimentation. Experience in a tropical developing country an advantage. The training of Indonesian scientists will be an important responsibility and the successful applicant will be expected to show an interest and aptitude in this area.

SALARY: Research Scientist/Senior Research Scientist: $A15,916 to $A23,122 p.a.

TENURE: Subject to negotiation, but a minimum of 3 to 4 years is envisaged. The appointee may be offered an indenfinite appointment within CSIRO.

CONDITIONS: Overseas conditions of service apply with respect to housing and allowances. As training of local staff is an integral part of the duties, it is essential that the successful applicant attain a degree of fluency in Indonesian language. To this end, a language course will be arranged.

Applications IN DUPLICATE, stating full personal and professional details, the names and addresses of at least two professional referees, and QUOTING REFERENCE NUMBER 1000/50 should reach:-

The Personnel Officer, Australian Scientific Liaison Office, Canberra House, 10-16 Maltravers Street, London WC2R 3EH, by 12th October, 1979.

Applications in U.S.A. and Canada should be sent to:- The Counsellor Scientific, Embassy of Australia, 1601 Massachusetts Avenue, N.W., Washington, D.C., 20036, U.S.A. 3201(A)

STANFORD UNIVERSITY

GEOCHEMIST —PETROLOGIST

The Department of Geology in the School of Earth Sciences invites applications for a faculty position beginning September, 1980. Areas of interest include (but are not limited to) geochemical processes in the crust and mantle of the earth including isotope geochemistry, thermodynamics, kinetics, element distribution and phase equilibria studies. Teaching responsibilities might include graduate and undergraduate courses in geochemistry and igneous petrology. Level of appointment is open, but we are particularly interested in a creative young person, with a well-established record in research.

Applications should include (1) a resume of education, professional experience, and published research; (2) a brief statement of plans for future research, and (3) the names of 4 referees. Send to: Prof. J.G. Liou, Department of Geology, Stanford University, Stanford, CA 94305, by December 1st, 1979. Stanford University is an equal-opportunity/affirmative-action employer. W329(A)

UNIVERSITY OF DURHAM

DEPARTMENT OF APPLIED PHYSICS AND ELECTRONICS

LECTURER IN THE DEPARTMENT OF APPLIED PHYSICS AND ELECTRONICS

Applications are invited for the post of Lecturer in the Department of Applied Physics and Electronics tenable from 1st January 1980 or as soon as possible thereafter.

Salary will be at an appropriate point on the scale £4,333 to £8,992 plus superannuation benefits, according to age, qualifications and experience.

Candidates should have research and/or industrial experience in an area that will extend and complement the present Departmental interests in solid state physics and in electronics.

Applications (three copies) naming three referees should be submitted to the Registrar and Secretary, Science Laboratories, South Road, Durham DH1 3LE, not later than Monday, 22nd October, 1979. 3195(A)

British Museum (Natural History) London

Protozoologists

The British Museum (Natural History) is an institution for taxonomic research and an educational focal point for the public. The main aim of the research is to produce definitive accounts of the world's animals, plants and minerals as well as the preservation of an extensive collection of fossils.

RESEARCH ON CILIATED PROTOZOA

This post offers an experienced protozoologist the opportunity to carry out specialist taxonomic studies on marine benthic ciliated protozoa. There are excellent facilities for experimental culture work and light and electron microscopy.

Candidates, normally aged under 32, should have a good honours degree or equivalent in a relevant biological science. At least 2 years' postgraduate experience is required, part of which must be in protozoology.

Appointment as Senior Scientific Officer £6,655 to £8,640 or Higher Scientific Officer £5,505 to £6,990; (to become £7,110 to £9,485 and £5,875 to £7,515 respectively from 1.1.80). Level of appointment and starting salary according to qualifications and experience. Promotion prospects. Non-contributory pension scheme.

TAXONOMIC RESEARCH ON FRESHWATER CILIATED PROTOZOA

As a member of a small research team the successful candidate will be actively involved with taxonomic studies on freshwater ciliated protozoa.

Candidates, normally aged under 27, should have a good honours degree or equivalent in zoology or microbiology or related discipline. Some experience of protozoology advantageous.

Appointment as Scientific Officer. Starting salary (according to qualifications and experience) within the range £4,065 to £5,865 (to become £4,370 to £6,265 from 1.1.80). Promotion prospects. Non-contributory pension scheme.

For further details and an application form (to be returned by 5th October 1979) write to Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, or telephone Basingstoke (0256) 68551 (answering service operates outside office hours). *Please quote ref: SB/61/DK.* *3172(A)*

RESEARCH OFFICER

Department of Chemistry

(Ref: CH/157)

NIBSC is an Institute concerned with the standardisation and control of biological substances used in medicine and is a WHO laboratory for biological standards. The work of the Institute is well supported by research.

The post is in the laboratory concerned primarily with the chemical and physical analysis of peptides and enzymes. A range of techniques is employed, including uv and fluorescence spectroscopy, all forms of chromatography and electrophoresis, and enzyme assay procedures. Applicants should have a degree in an appropriate discipline. Experience in some of the techniques mentioned would be advantageous.

Salary, depending upon age and experience, on the scale £3,715 to £5,259 p.a. (pay award pending), including London Weighting, plus superannuation benefits.

Please write or telephone for further details and an application form, quoting reference CH/157, to the Personnel Officer, NIBSC, Holly Hill, Hampstead, London NW3 6RB (Tel: 01-435 2232, Extension 200). 3188(A)

NIBSC

National Institute for Biological Standards and Control

UNIVERSITY OF BRISTOL

SCHOOL OF VETERINARY SCIENCE

A POSTGRADUATE RESEARCH WORKER

is required to join a group working on mucosal immunity in the Department of Animal Husbandry, Langford. The major study will be the development of hypersensitivity mediated tissue damage following oral presentation of antigens.

The project, funded by the Agricultural Research Council, is for a three year period, leading to a higher degree. Salary will be based on University scales.

Applications, giving the names and addresses of two referees, should be sent to The Professor of Animal Husbandry, Langford House, Langford, Bristol BS18 7DU. 3190(A)

UNIVERSITY OF QUEENSLAND

Australia

LECTURER IN PHARMACY

Must qualify for pharmaceutical registration and have appropriate higher degrees. Should have proven ability in Clinical Pharmacy and Pharmacy Practice. Will be responsible for teaching Clinical and Professional Pharmacy. Also liaison between Pharmacy, Medicine and teaching hospitals in Brisbane, and general administration. 8.10.79.

Salary: $A16,291 to $A21,401 p.a. Other Benefits: Opportunities to undertake programmes of special study, superannuation, housing assistance, travelling and removal expenses.

Additional information and application forms are obtainable from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

3147(A)

DEPARTMENT OF CHILD HEALTH

UNIVERSITY OF MANCHESTER

RESEARCH FELLOW IN ANATOMY

Postdoctoral Research Fellow required in multidisciplinary research team to organise new work in quantitative histology, mainly but not exclusively in the brain.

Enquiries with full curriculum vitae to Professor John Dobbing, The Medical School, Oxford Road, Manchester M13 9PT 3197(A)

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY

South Kensington, SW7

RESEARCH TECHNICIAN GRADE 5

Duties include the operation and maintenance of analytical equipment, and the provision of assistance and advice in the use of research instruments.

Applicants must have considerable experience in the use of instruments appropriate to biological sciences, and preferably be qualified to HNC or advanced 1ST/CG standard.

Salary in range £4,224 to £4,844 inclusive (under review).

The post is superannuable. There is a generous sick pay scheme. The working week is 37 and a half hours. Five weeks annual holiday plus several days in addition to public holidays at Christmas and Easter Modern staff club and excellent facilities with sports centre and swimming pool Season ticket loan scheme after 6 months

Apply, in writing, to Mr C.L. Meredith, Departmental Superintendent, Zoology Department, Imperial College, SW7 2BB or telephone 01-589 5111 extension 2263.

3182(A)

CHARING CROSS HOSPITAL MEDICAL SCHOOL

(University of London)

LECTURER IN DEPARTMENT OF CHEMICAL PATHOLOGY

Applications are invited for the above post from either medically or non-medically qualified persons. Responsibilities include teaching and research and some health service duties for which it is expected that an appropriate honorary NHS contract will be granted.

Salary on scale £4,986 to £7,393 (under review) plus £354 London Weighting Allowance (medically qualified) or £4,333 to £8,992 plus £740 London Weighting Allowance (for those without a medical qualification).

Applications in writing with names and addresses of two referees or further information and application forms if required obtainable from The Secretary, Charing Cross Hospital Medical School, The Reynolds Building, St Dunstan's Road, London W6 8RP to be submitted by 12th October, 1979. (Ref: 050/2)

3178(A)

UNIVERSITY OF OTAGO

Dunedin, New Zealand

CHAIR OF EXPERIMENTAL ORAL BIOLOGY

The Council of the University of Otago invites applications for appointment to the foundation Chair of Experimental Oral Biology in the Department of Oral Biology in the Faculty of Dentistry, University of Otago.

Professorial salaries are fixed at various points within a present range from NZ$23,865 to $33,865 per annum according to whether the appointee holds dental, medical or other qualifications; increases are pending.

Further particulars are available from the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or from the Registrar, University of Otago, P.O. Box 56, Dunedin, New Zealand. Applications close on 31 October, 1979.

3183(A)

UNIVERSITY OF BRISTOL

SCHOOL OF CHEMISTRY

GRADUATE RESEARCH ASSISTANT

Applications are invited from plant biochemists or plant biologists for this post to undertake research into the identification, photo-control of levels, and metabolism of gibberellins in plant leaves and etioplasts. The post which is funded by ARC is tenable for three years at a salary of £3,775 to £4,333 (Scale effective from 1st October, 1979 and subject to further review). The successful candidate will be encouraged to register for a higher degree.

Applicants should send a curriculum vitae and names of referees as soon as possible, to Professor J. MacMillan, School of Chemistry, The University, Bristol BS8 1TS. 3164(A)

THE UNIVERSITY OF SYDNEY

LECTURESHIPS (2) IN PHARMACOLOGY

Appointees will be responsible for conduct and development of teaching and research in neurochemistry and neuropharmacology in Department of Pharmacology. Research interests in mammalian central nervous system are desirable and willingness to participate in collaborative multidisciplinary research with Professor G.A.R. Johnston on amino acid neurotransmitters would be welcomed.

The positions are expected to be filled by probationary appointments of three years capable of leading to tenure, but if all the University's requirements for tenure are deemed to be satisfactorily met, tenure may be granted at time of appointment.

Salary range: $A16,291 to $21,401 p.a.

Applications including list of publications, curriculum vitae and names of three referees by *19 October 1979* to the Registrar, University of Sydney, NSW 2006, Australia, from whom further information available. Information also available from Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. 3184(A)

MEDICAL RESEARCH COUNCIL

Newcastle Upon Tyne

NEUROENDOCRINOLOGY UNIT

NEUROCHEMIST

An experienced neurochemist is required to join the recently established MRC Neuroendocrinology Unit at Newcastle General Hospital. The person appointed will be expected to lead a group working on the biochemical properties of peptidergic neurones, their interactions with other types of nerve-cell and possible involvement in clinical disorders of the nervous system. The post will be available from April 1980, and appointment will be to the Council's non-clinical Scientific Staff at Grade I (£6,757 to £8,452) or, in the case of an exceptional candidate, an appointment at Senior Grade (£8,182 to £10,097) would be considered.

Potential applicants should write, in the first place, to the Director, Dr J A Edwardson, at the MRC Neuroendocrinology Unit, Newcastle General Hospital, Westgate Road, Newcastle upon Tyne, NE4 6BE. 3198(A)

MEDICAL LABORATORY Scientific Officer required in Bacteriology Department for research in development of automated techniques for identification of bacteria using enzyme assays. Some experience in bacteriological and/or biochemical techniques preferred. Post is grant-funded for initial 2 years. Applicants should have minimum HNC, AIMLS, BSc or equivalent. Salary within scale £3,357 to £4,266 + £354 p.a. London Allowance. Apply with full curriculum vitae and names of 2 referees to The Secretary, St. Mary's Hospital Medical School, Paddington, London W2 1PG quoting Ref MLSO/SB/B/N. 3166(A)

KING'S COLLEGE HOSPITAL MEDICAL SCHOOL

(University of London)
Denmark Hill, London
SE5 8RX

JUNIOR MEDICAL LABORATORY SCIENTIFIC OFFICER 'B'

required for the Chest Unit in this Medical School. Work will involve contact with patients and assisting doctors with research into diseases of the lung.

Minimum requirements, 5 'O' levels, 4 in Science subjects including maths, day release available. Post suitable for school leaver.

Salary according to age, qualifications and experience.

Applications, in writing, giving full details should be sent to the Secretary of the Medical School at the above address. Closing date 28th September, 1979. 3181(A)

CSIRO AUSTRALIA

Research Scientist

Division of Animal Production Sydney

Field: Endocrine Control of Mineral Metabolism.

CSIRO has a broad charter for research into primary and secondary industry. The Organization has approximately 7,000 employees — some 2,400 of whom are research and professional scientists — located in Divisions and Sections throughout Australia.

The Ian Clunies Ross Animal Research Laboratory is the Headquarters of the Division of Animal Production and is situated at Prospect, 20 miles west of Sydney NSW.

General: The Division has six broadly-based programmes of research on tropical animal production, strategic nutrition of livestock, skin and fleece biology, mineral requirements for livestock production, improvement in reproductive efficiency, and animal breeding and genetics, and has laboratories in Rockhampton, Armidale, Sydney and Perth.

Duties: These would involve the initiation of research projects dealing with the metabolism of phosphorus and calcium in ruminants (sheep and cattle). Emphasis will be placed on mechanisms controlling the movement of calcium and phosphorus within and between the various compartments in the body. This could embrace investigation into the hormonal regulation of intestinal absorption, bone resorption, salivary secretion and renal excretion. Developmental changes in these processes should also be considered.

Qualifications: PhD degree in an appropriate field or equivalent, with experience in the field of phosphorus and calcium metabolism in ruminants. A thorough biological knowledge should be combined with experience in tracer and compartmental analysis.

Salary: Research Scientist or Senior Research Scientist: $A15,916 to $A23,122 p.a. An outstanding applicant may be offered appointment at a higher level.

Tenure: Indefinite with superannuation.

Location: The position will be located at Prospect, initially, but may later be moved to Rockhampton, Queensland.

Applications in duplicate, stating full personal and professional details, the names and addresses of at least two professional referees, and quoting reference number 245/727 should reach: —

The Personnel Officer, Australian Scientific Liaison Office, Canberra House, 10-16 Maltravers Street, London WC2R 3EH, by 12th October, 1979.

Applications in U.S.A. and Canada should be sent to: The Counsellor Scientific, Embassy of Australia, 1601 Massachusetts Avenue, NW, Washington, DC, 20036, U.S.A.

3168(A)

JUNIOR TECHNICIAN

Department of Antibiotics

(Ref: AB/007)

to work in a laboratory concerned with the standardisation and control of antibiotics used therapeutically and to assist in the development of suitable analytical methods.

Candidates should possess or be studying for an HNC in one of the biological sciences. Microbiological laboratory experience would be an advantage.

Salary, depending on qualifications and experience, on the scale £2,892 to £5,034 (pay award pending) including London Weighting, plus superannuation benefits.

Please write or telephone for further details, and an application form, to the Personnel Officer, National Institute for Biological Standards and Control, Holly Hill, London NW3 6RB (telephone 01-435 2232, ext 200), quoting Reference No. AB/007.

NIBSC

National Institute for Biological Standards and Control

3187(A)

Microbiologist

Key involvement in advanced foods research

£10,000 + benefits Midlands

A substantial multi-million annual research budget is a clear indication of the strong commitment of our client to the development of new or improved products to meet the changing demands of the marketplace. It's a policy which has placed the company at the very forefront of food microbiology technology, one which at the same time affords considerable scope for original and challenging work in a progressive research environment.

With a minimum of 2 years' research experience and ideally an interest in the industrial applications of microbial ecology, you will be heading key research projects in the preparation and preservation of foodstuffs. As well as the more formal aspects of microbiological research this includes liaison with universities, research establishments and other professional bodies, and you will be providing a specialist consultancy service to other members of the R & D team.

Aged 26-32, you should have a good honours degree — preferably a PhD. A knowledge of microbial ecology, food microbiology or preservation technology would be an advantage. *Ref: W4936/N*

Replies will be forwarded direct, unopened and in confidence to the client unless addressed to our Security Manager listing companies to which they may not be sent. They should include comprehensive career details not refer to previous correspondence with PA and quote the reference on the envelope.

3207(A)

PA Advertising

Hyde Park House, 60a Knightsbridge, London SW1X 7LE. Tel: 01-235 6060 Telex: 27874

PA

A member of PA International

POST-DOCTORAL RESEARCH ASSISANT required in Department of Microbioloy to work on a three-year M.R.C. project on latency of human papovaviruses. Suitable for biochemist or virologist with experience in nucleic acid research. Salary not less than £4,262 plus £502 London Allowance for suitably qualified person. For further information telephone Dr McCance (01-407 7600 ext. 3376). Apply in writing, with full curriculum vitae, to the Secretary, Guy's Hospital Medical School, London Bridge, SE1 9RT, quoting Ref. M.C.5. 3078(A)

UNIVERSITY OF SHEFFIELD
ELECTRON MICROSCOPY TECHNICIAN (Grade 5)
required for the
DEPARTMENT OF HUMAN BIOLOGY & ANATOMY

Applicants should have experience in the operation, and at least basic maintenance of transmission electron microscopes. Experience in biological specimen preparation, while valuable, is not essential. Salary on scale £3,700 to £4,320 p.a.

Please send details of qualifications, experience and the names of two referees to the Administrative Officer (Personnel), (Ref. S1362/N), or write informally to Professor R. Barer, Department of Human Biology and Anatomy, the University, Sheffield S10 2TN. 3191(A)

POST-DOCTORAL POSITION available involving research in DNA replication in mammalian cell-virus systems. Salary: $16,000. Please send Curriculum Vitae to: Mrs Lucy Luckhardt, Sloan-Kettering Institute, Walker Laboratory, 145 Boston Post Road, Rye, NY 10580. W354(A)

UNIVERSITY OF LEICESTER
DEPARTMENT OF BIOCHEMISTRY
POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for a postdoctoral research assistantship supported by the MRC to work with Dr D Critchley on cell surface changes and malignancy. The project is directed toward investigating the possible relevance of loss of complex glycolipids to other aspects of the malignant phenotype, including loss of fibronectin. Experience in cell culture and membrane biochemistry is desirable but not essential.

The appointment will commence as soon as possible; the post is most suited to candidates with at least 1 or 2 years postdoctoral experience, although recently qualified candidates will be considered. The salary will commence in the range of £4,333 to £5,199 depending on the candidate's age and experience.

Applications including curriculum vitae and the names of two referees should be sent to Dr D Critchley, Department of Biochemistry, University of Leicester, Leicester LE1 7RH. 3173(A)

LABORATORY SCIENTIFIC OFFICER required in Department of Microbiology to work on a three-year M.R.C. project on latency of human papovaviruses. Experience in biochemical aspects of virology essential. Salary for qualified Laboraory Scientific Officer not less than £3,261 (under revision) plus £354 London Weighting. Apply in writing, stating age and giving details of qualifications and experience, to the Secretary, Guy's Hospital Medical School, London Bridge, SE1 9RT, quoting Ref. M.C.4. 3077(A)

UNIVERSITY OF DUNDEE

Applications are invited from candidates who hold a good Honours degree in Biochemistry or related subject for appointment as a

RESEARCH ASSISTANT

in the University Department of Surgery at Ninewells Hospital and Medical School. The successful applicant will join a team investigating certain biochemical aspects of breast cancer. The appointment is supported by a SHERT grant and will be available for three years from 1st October, 1979 or some other agreed date. Initial salary at an appropriate point within the range £3,775 to £4,333. Superannuation under USS. Possibility of registration for a higher degree.

Applications, quoting Reference EST/81/79J and containing full details of qualifications and experience, and the names of three referees, should be sent to The Secretary, The University, Dundee DD1 4HN by 5th October 1979. Informal enquiries may be made to Dr P.R. Baker, Department of Surgery, Ninewells Hospital and Medical School, Dundee. 3153(A)

PLYMOUTH POLYTECHNIC
SCHOOL OF ENVIRONMENTAL SCIENCES
RESEARCH ASSISTANT IN SEDIMENTOLOGY/PALAEOECOLOGY

To investigate the lithostratigraphy, biostratigraphy, sedimentology and palaeoecology of the Upper Greensand (Cretaceous).

Intending applicants should have a good honours degree in the geological sciences.

Research assistants are normally required to register for a higher degree, although post doctoral applicants will be considered.

Appointments are for a period of two years, with a possibility of a third year (fixed term contracts). **Salary will be £3,480 for postgraduates or £3,780 post-doctoral (or equivalent) with annual increments of £250.** Application forms, returnable by *5 October 1979*, together with further particulars, from the **Personnel Officer, Plymouth Polytechnic, Drake Circus, Plymouth PL4 8AA.** 3179(A)

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE
ACADEMIC DEPARTMENT OF PHARMACOLOGY
POST-DOCTORAL RESEARCH FELLOW IN RESPIRATORY PHARMACOLOGY

A post-doctoral Research Fellow with a physiological or pharmacological background is required to work on the actions of adrenoceptor blocking drugs on the lung. Training in *in vivo* techniques for measuring lung mechanics will be given.

Salary from £4,333 per annum plus £740 London Allowance.

For further details please contact Dr J Maclagan, Academic Department of Pharmacology, RFHSM, Clinical Sciences Building, Pond Street, NW3 2QC.

The closing date for applications is 12 October, 1979. 3194(A)

The Queen's University of Belfast
RESEARCH ASSISTANTS
Department of Applied Mathematics and Theoretical Physics

Research Assistant posts funded by the Science Research Council are available at both postdoctoral and postgraduate level for research in Atomic and Molecular Physics. The posts are tenable for both two and three year periods commencing 1st October 1979, or as soon as possible thereafter. Successful applicants will participate in the theoretical study of atomic collision processes of relevance in astrophysics, laser physics and fusion physics.

Applicants must have a good honours degree in Physics or Applied Mathematics and some experience of the practical use of computers. A knowledge of Atomic collision physics would be an advantage.

Commencing salary will be: Postdoctoral: £4,911; Postgraduate — range: £3,777 — £4,335.

Curriculum vitae, giving the names and addresses of two referees should be sent to the Personnel Officer, The Queen's University of Belfast, BT7 1NN, Northern Ireland. Closing date: 28th September, 1979. 3152(A)

CSIRO AUSTRALIA

Postdoctoral Research Fellow

Division of Land Resources Management Perth

Field: Physics and Chemistry of Forest Soils.

CSIRO has a broad charter for research into primary and secondary industry. The Organization has approximately 7,000 employees — some 2,400 of whom are research and professional scientists — located in Divisions and Sections throughout Australia.

General: The Division does research for the better management and husbanding of land and water resources. This includes environmental and societal implications of resource management in pastoral, agricultural, forested and near urban areas. One research programme based in Perth, W.A. is specifically studying the effect of using forested lands on the future productivity and conservation of forest resources. The appointee would be a member of this group.

Duties: To initiate research into the environmental consequences of intensive forest harvesting, in particular the effects of site characteristics and harvesting practices on soil compaction, erosion, and sediment and nutrient transport.

Qualifications: PhD or equivalent in an appropriate field of study.

Salary: Research Scientist: $A15,916 to $A19,509 p.a.

Tenure: 5 years.

Applications in duplicate, stating full personal and professional details, the names and addresses of at least two professional referees, and quoting reference number 284/102 should reach:—

The Personnel Officer, Australian Scientific Liaison Office, Canberra House, 10-16 Maltravers Street, London WC2R 3EH, by 12th October, 1979.

Applications in U.S.A. and Canada should be sent to: The Counsellor Scientific, Embassy of Australia, 1601 Massachusetts Avenue, NW, Washington, DC, 20036, U.S.A.

3169(A)

UNIVERSITY OF BRISTOL

Long Ashton Research Station

REQUIRED IN THE FOOD AND BEVERAGES DIVISION:—

POST A — MICROBIOLOGIST OR MICROBIAL BIOCHEMIST to investigate the effect of permitted preservatives and naturally occurring antimicrobial factors on the growth of wine spoilage yeasts and bacteria.

POST B — NATURAL PRODUCTS CHEMIST to investigate the formation of polymeric pigments from anthocyanins and phenolics and their stability in wines. Research with purified components in model systems to determine the causes of instability will be correlated with structural studies of the polymers.

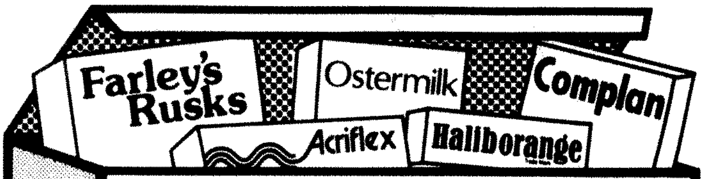
POST C — NATURAL PRODUCTS CHEMIST to investigate the formation of polymeric pigments from anthocyanins and phenolics in fortified wines. Recent work shows such polymers can affect maturity, quality and resistance to microbial spoilage.

Minimum qualifications: 2(1) Honours degree or equivalent; Post A in microbiology or microbial biochemistry; Posts B and C in natural products chemistry. At least two years' postgraduate experience, preferably with a higher degree, or equivalent experience required for all 3 posts.

Posts A and B financed by industry on a long term basis. Post C financed by industry for 3 years initially with a possibility of an extension for a fourth. All in the Higher Scientific Officer grade (£4,727 p.a. rising to £5,097 p.a. from 1.1.80 to a maximum of £6,737 p.a.). Starting salary according to qualifications and experience. Non-contributory pensions scheme.

Further particulars and application forms from the Secretary, Long Ashton Research Station, Long Ashton, Bristol, BS18 9AF, to whom applications should be sent by 5th October, 1979.

3185(A)



Farley Health Products Ltd.

Technical Information Officer

Devon **£6000 +**

Farley Health Products wish to appoint a Technical Information Officer based at their Plymouth headquarters. This is a new appointment and the successful applicant will be expected to establish efficient information storage and retrieval systems, monitor relevant sources, carry out literature surveys, be responsible for the technical library facilities and contribute a technical information input into the product development, quality control, marketing, sales and production functions.

Farley Health Products Ltd, as part of the Glaxo Group, market a range of infant and dietetic foods and over-the-counter medicines. Considerable food and pharmaceutical process and product development programmes are currently in progress and the person appointed will be expected to make a significant contribution to this work. Liaison with the information services in the other Glaxo Group companies will also be involved.

Applicants, either male or female, should be of graduate standard and preferably have A.L.A./F.L.A. or equivalent qualifications and proven ability in similar work.

Excellent conditions of employment include an opportunity to participate in the Group's profitability, life assurance and pension schemes. Assistance with relocation is available where appropriate.

Please write or phone for application form to:-

Dr. L. F. J. Parker, Farley Health Products Ltd.,
Torr Lane, Plymouth, Devon PL3 5UA.
Telephone No: (0752) 701621.

A Glaxo Group Company 3171(A)

THE UNIVERSITY OF SHEFFIELD

DEPARTMENT OF PHYSIOLOGY

Applications are invited from men and women science or medical graduates for

TWO LECTURESHIPS

in the above Department. For one post, applicants should have research experience in the neurophysiology of the gastrointestinal tract and for the other, experience in cardiovascular and respiratory systems or in endocrinology. Initial salary in range £4,333 to £5,199 a year on scale rising to £8,992 a year. Those interested should contact Professor T. Scratcherd, Department of Physiology, for further details.

Applications (5 copies), including the names and addresses of three referees, to the Registrar and Secretary, the University, Sheffield S10 2TN by 5 October, 1979. Quote ref. R362/G. 3189(A)

THE ROYAL FREE HOSPITAL

HAEMOPHILIA CENTRE AND HAEMOSTASIS UNIT

Research Assistant (Basic Grade Biochemist) to work on the development and application of new assay methods which will be used in the investigation and management of patients with bleeding and thrombotic disorders. Experience with radioimmunoassay would be an advantage.

Salary on scale £4,035 to £4,935 p.a. plus £354 London Weighting. The post is for 1 year in the first instance.

Applications or enquiries should be directed to Dr P.B.A. Kernoff, Haemophilia Centre, Royal Free Hospital, Pond Street, London NW3 2QG. Tel: 01-794 0500 ext 4140. 3196(A)

INFORMATION PUBLISHING

GRADUATE PHYSICAL OCEANOGRAPHER

with a knowledge of marine geology, required to work on the editorial team of Aquatic Sciences and Fisheries Abstracts, Part 2: Ocean Technology, Policy and Non-Living Resources. Starting salary £3,600 rising to £4,032 after one year. Please apply in your own handwriting, enclosing full curriculum vitae and stating when you would be free to start to:

Carolyn Roberts
Managing Editor
Information Retrieval Limited
1 Falconberg Court
London W1V 5FG

IRL®

3186(A)

Biochemist -Development Role around £6500

Due to further expansion, we need an able versatile life scientist to play an important role in development work associated with the production of our clinical assay systems. This new position calls for strong theoretical and practical skills as the work will involve many aspects of new product development and trouble-shooting.

Protein chemistry, radiolabelling techniques, freeze-drying methodology and assessment of new plant and equipment are all important features of the work.

This position will appeal to life science graduates who have had further experience and/or research training either in clinical chemistry or allied areas of biochemical research. It could provide an interesting career move to a research scientist wishing to broaden his/her development interests within a production environment.

The Company is based at Amersham but has a completely new production facility under development at Cardiff and this post will transfer to Cardiff during 1980. Our continued growth as an international organisation is reflected by a turnover of around £40m per annum and our increasing influence in a wide range of world markets.

Attractive benefits package includes assistance with relocation expenses, where appropriate.

Please telephone Dr. Stewart Allinson on Little Chalfont (02404) 4444, ext. 3908 for an application form, or write to him with concise career details at:

3202(A)

The Radiochemical Centre
Amersham Bucks

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universit college o swansea

Histology Technician

Applications are invited for the vacancy of **Histology/Micotomy Technician, Grade 5,** in the **Department of Zoology.** The duties of the post will include processing tissues, cutting serial sections, staining and mounting slides for teaching and research purposes, sharpening microtome blades, caring for microscopes and allied equipment, together with some general laboratory work.

The salary will be on the scale £3,700 to £4,320 per annum.

Application forms may be obtained from the Personnel Officer, University College of Swansea, Singleton Park, Swansea, SA2 8PP, to whom they should be returned by *Tuesday, 25th September, 1979.* 3200(A)

British Nutrition Foundation

The BNF combines the expertise of leading medical, academic, governmental and industrial experts in its educational and research activities.

There are two vacancies for persons of high ability:

SENIOR SCIENTIFIC OFFICER (SSO)
SCIENTIFIC INFORMATION OFFICER (SIO

The SSO will have a good honours degree and several years relevant post-graduate experience. The varied work will include 'in depth' studies and the preparation of reports for publication.

The SIO will have a good honours degree or other relevant qualification and experience, and must be capable of explaining matters relating to food, nutrition and health in simple terms, by written and spoken word to health and education professionals and to lay persons.

Applications should reach the Secretary, British Nutrition Foundation, 15 Belgrave Square, London SW1, before Monday 1 October 1979.

3205(A)

PLYMOUTH POLYTECHNIC
School of Maritime Studies
RESEARCH ASSISTANT IN ASTRONOMY

To analyse data connected wit magnetic field of the Milky Way. Ar interest in computing and astronomica data analysis is essential. Applicant should have a good honours degree i Physics, Mathematics or Astronomy. Register Assistants are normall required to register for a high degree although post doctoral applicants wi be considered.

Appointments are for a period of years, with a possibility of a 3rd yea (fixed term contracts). *Salary will b £3,480 for Post Graduates or £3,78 Post Doctoral (or equivalent) wit annual increments of £250.*

Application forms, returnable by *12t October 1979* and further particulars to *Personnel Officer, Plymout Polytechnic, Drake Circu Plymouth, Devon PL4 8AA.* 3209(A

INSTITUTE OF DERMATOLOGY

(University of London)

SENIOR TECHNICIAN

required to assist in the running of a VG 305/2015 Mass Spectrometer/Data System. Experience in the field of quantitative organic gc-ms would be an advantage. Salary within the range of £5,370 to £6,939 (inclusive of London weighting).

Applications in writing, enclosing a c.v. to Professor M.W. Greaves, Institute of Dermatology, Homerton Grove, London E9 6BX. **3199(A)**

ROYAL HOSPITAL FOR SICK CHILDREN
Edinburgh

DEPARTMENT OF PATHOLOGY

POSTDOCTORAL RESEARCH FELLOW

required for work on the expression of enzyme markers in milignant disease. The appointment is for one year in the first instance and the starting salary will be £4,622 (university range 1A).

Applications giving the names of two referees should be sent as soon as possible to:

The Secretary, Department of Pathology, Royal Hospital For Sick Children, Edinburgh, EH9 1LF. Tel. 031 667 1991 ext 270. 3206(A)

ASSISTANT: STRUCTURAL GEOLOGY, ETH Zürich

Applications are invited for a position as Assistant to Professor Ramsay for work on a research project "Tectonic and geophysical studies of the Helvetic Alps" supported by the Swiss National funds.

Applicants should hold a PhD or equivalent degree. The work will consist of field mapping of large and small scale tectonic structures, strain analysis, and laboratory studies of deformed Mesozoic and Tertiary sediments. An x-ray texture goniometer and Philips TEM 400 are available for this study. The position starts October 1 1979 and will end May 31 1981.

Further information will be sent on request. Applicants should send a curriculum vitae and the names of two references to Professor J.G. Ramsay, Geol. Inst., ETH Zentrum, CH 8092 Zürich Switzerland. W356(A)

Australian Institute of Marine Science

Research Positions

The Australian Institute of Marine Science was established as a Statutory Authority of the Commonwealth Government in 1974 and, in 1977, took occupation of a modern and comprehensively equipped laboratory complex at Cape Ferguson, near Townsville, well situated for studies in tropical marine science including phenomena at the land-sea interface, on the complex environment of the Great Barrier Reef and in the adjacent ocean. The ongoing programs of the Institute are augmented by active collaboration and interaction with visiting investigators. Staffing of the Institute is expanding to match developing commitments in comprehensive and coherent studies of the Great Barrier Reef environment, its living communities and their dynamic behaviour. Enquiries are invited from professional researchers interested in appointment to participate in such an investigation. Expertise is sought in the following fields: Physical, Chemical and Biological Oceanography, Reef Metabolism, Community and Population Ecology.

Duties: The appointees will be expected to join effectively in a group concerned at developing a fundamental understanding of the environment of the Great Barrier Reef and the ecology of its living communities including their organization, component interactions stability characteristics.

Qualifications: The appointee must possess PhD level qualifications and preferably experience demonstrably applicable to the research problem outlined. Levels of appointment area open. Tenure will be offered if appropriate. However, those who might wish collaboration for a fixed term are also encouraged to indicate their interest.

Applications should be accompanied by a complete C.V., the names of at least 3 but no more than 5 referees and should be forwarded to the address given below no later than 31 October 1979.
The Secretary, Australian Institute of Marine Science, P.M.B. No.3, M.S.O. Townsville, O. 4810, Australia. 3170(A)

UNIVERSITY OF AARHUS

Denmark

A one year temporary position as adjunkt/lektor (associate professor or assistant professor according to qualifications) with possibility of extention is vacant at the Department of Molecular Biology and Plant Physiology starting January 1, 1980.

The successful applicant will be required to work in the area of symbiotic nitrogen fixation in higher plants as part of a group within the department. The research will primarily deal with the molecular genetics of Rhizobium. Applicants with research experience in bacterial and molecular genetics will be preferred.

The successful applicant must, on equal terms with the other members of the staff, share the teaching responsibilities — from first-year level to supervision of graduate and post-graduate students. Further information can be obtained from professor K.A. Marcker, phone Denmark (06) 12 51 77.

Applications containing curriculum vitae, information on scientific and educational activities, a list of publications plus wherever possible 3 copies of published material, and any additional information relevant for the evaluation of the applicant's scientific and educational qualifications should be sent to the Faculty of Science, University of Aarhus, Ndr. Ringgade 1, DK-8000 Århus C, Denmark, before October 7, 1979.

The report of the evaluation committee will be sent to all applicants. W355(A)

GLASSHOUSE CROPS RESEARCH INSTITUTE

requires

SCIENTIFIC LIAISON OFFICER.

Duties include arrangements for and reception of visitors, assisting in organising Open Days, preparation and presentation of exhibits, answering queries, assisting in preparation of publications. Appointee should have sound knowledge of horticulture.

Appointment in Higher Scientific Officer grade, salary £4,727 to £6,211 p.a. (£5,097 to £6,737 p.a. from 1 January 1980). Starting salary depending on qualifications and experience. Applicants should have Honours degree in relevant scientific subject with at least three years post-graduate experience. Non-contributory superannuation scheme. Further details and application forms on request.

Applications to Secretary, Glasshouse Crops Research Institute, Worthing Road, Rustington, Littlehampton, West Sussex, BN16 3PU, by 1 October 1979. 3211(A)

The Research Unit of Organon Laboratories Limited, Newhouse is an integral part of the Scientific Development Group of Organon International a major international pharmaceutical company based in the Netherlands.

Applications are invited for the position of

Immunopharmacologist

in our Biochemistry R&D Laboratories. This department is responsible for both exploratory and developmental research directed towards the production of new therapeutic agents for the treatment of chronic inflammatory disease. The experimental approach is mainly directed towards agents modifying the immune system so the appointment offers a challenging opportunity to join a young and enthusiastic interdisciplinary team working in this rapidly developing area of research.
Suitable candidates will already have at least 2-3 years post-doctoral experience in the immunopharmacology of chronic inflammation and will show the ability to direct the research of a group of 2-3 scientists and technicians.
The successful candidate will be expected to maintain and develop his expertise and reputation in the field by attendance at scientific meetings and by publication of his reserach results.
The initial salary will be based on qualifications and experience and will be commensurate with the responsibilities of the position. Conditions of employment are in keeping with good modern practice and assistance with relocation expenses will be available in appropriate cases.
Applications should be addressed to the Personnel Manager, Organon Laboratories Limited, Newhouse, Lanarkshire, ML1 5SH.

Organon

3154(A)

Please mention

Nature

when replying to these advertisements

UNIVERSITY OF LONDON

British Postgraduate Medical Federation

CARDIOTHORACIC INSTITUTE

(associated with the National Heart & Chest Hospitals)

CARDIAC METABOLISM RESEARCH LABORATORIES

50 Wimpole Street,
London W.1.

A vacancy exists for a

RESEARCH ASSISTANT

to assist in a project concerned with the identification and mechanisms of beta-receptors in myocardial tissue. The project is suitable for a PhD thesis and registration for this higher degree will be encouraged.

Applicants should have a degree in Biochemistry (first or upper second class). The post is offered for a maximum of three years. Minimum salary £3,768 + £354 London Allowance (from 1.1.80: £4,125 + £354).

Application forms available on request from the House Secretary, Cardiothoracic Institute, 2 Beaumont Street, London W1N 2DX (telephone 01-486 3043). Closing date 5th October 1979.
(This is a re-advertisement and previous applicants should not re-apply). 3210(A)

FERMENTATION MICROBIOLOGIST

We are a large forest products R&D organisation, and are expanding our efforts on the conversion of wood residues to valuable products. Applicant should have a good honours degree, and preferably a PhD, in one of the biological sciences, together with specialised knowledge of fermentation processes, ideally in the area of biochemical engineering.

Duties will be to grow microorganisms on wood-based materials in laboratory fermenters, including continuous culture equipment. He will work closely with chemists on suitable pretreatments of the wood substrate and will be expected to devise techniques for screening, collecting and optimizing the yield of a variety of end products.

Salary open; fringe benefits generous. For further information please write to: Mr I B Flann, Forintek Canada Corp, 800 Montreal Road, Ottawa, Ontario, K1G 3Z5, Canada. W330(A)

FOR SALE and WANTED

FOR SALE: OPTOMAX (Micromeasurements) Modular Image Analyser including CPU, Power supply, Video Moniter, Flexidraw Module, Circular Variable Frame Module and Averaging Unit. Less than two years old; little used. Please contact: Ms Margaret Androu, Scott Polar Research Institute, Lensfield Road, Cambridge, CB2 1ER.

3158(L)

ASSISTANTSHIPS

UNIVERSITY OF GLASGOW

Kelvin Laboratory

POSTDOCTORAL RESEARCH ASSISTANTSHIP IN NUCLEAR STRUCTURE PHYSICS

Applications are invited for the above post based at the Kelvin Laboratory to work in close collaboration with a group of 6-8 physicists from the University of Edinburgh. This group is engaged in a variety of electro- and photo-induced nuclear reaction studies using the 30 MeV and 170 MeV electron accelerators at the Kelvin Laboratory.

The post is tenable for two years starting within six months of 1st October, 1979. Initial salary will be within Range 1A of the scales for Research and Analogous Staff (£4,333 to £5,488) depending on age and experience. Superannuation benefits will apply.

Applications, stating age, academic qualifications, research experience and giving the names of three referees, should be submitted not later than 12th October, 1979, to Dr. R.O. Owens, Department of Natural Philosophy, University of Glasgow, Glasgow, G12 8QQ, or to Dr D Branford, Department of Physics, University of Edinburgh, Edinburgh EH9 3JZ, from whom further details of the post may be obtained. In reply please quote Ref. No. 4507M. 3176(P)

KINGSTON POLYTECHNIC

SCHOOL OF CHEMICAL

AND PHYSICAL SCIENCES

POLYMER RESEARCH ASSISTANTSHIP

Opportunity for Honours graduate chemist to investigate synthesis of crosslinkable polymers, and determine how their structure affects their water resistance, using physical property measurements.

Successful candidate could register for PhD. Supervisor Dr G Pritchard. 3 year project. Starting salary £3,000-£3,500. Further details and application forms (To be returned as soon as possible) from Academic Registry, Dept. AO, Kingston Polytechnic, Penrhyn Road, Kingston upon Thames KT1 2EE. 01-549 1366.
3157(P)

UNIVERSITY OF GLASGOW

DEPARTMENT OF
CELL BIOLOGY

POST-DOCTORAL RESEARCH ASSISTANT

Applications are invited for a research assistantship, funded by the M.R.C., to study the membrane properties of mononuclear phagocytes as related to their behaviour. The person appointed would work with a small group interested in the cellular aspects of inflammation; a background in cellulr immunology or cell biology would be advantageous. The appointment is for a maximum of three years, starting on 1st October, 1979 or shortly thereafter, and would be on salary range 1A for Research and Analogous Staff (£4,333 to £5,777). Appropriate superannuation scheme will apply.

Applications, enclosing a curriculum vitae and the names of two referees, should be sent to Dr J.M. Lackie, Department of Cell Biology, University of Glasgow, Glasgow, G12 8QQ.

In reply please quote Ref. No. 4506M
3151(P)

UNIVERSITY OF GLASGOW

POSTDOCTORAL RESEARCH ASSISTANT IN NUCLEAR STRUCTURE PHYSICS

Applications are invited for a Research Assistantship at the Kelvin Laboratory in the Department of Natural Philosophy. The person appointed will participate in the nuclear structure research programme using the 170 MeV electron linear accelerator at the Laboratory.

The post is tenable until 31st January, 1982, when the present grant terminates. An early starting date would be desirable. Initial salary will be within Range 1A of the scales for Research and Analogous Staff (£4,333 to £5,488), depending on age and experience. Superannuation benefit will apply.

Applications, stating age, academic qualifications, research experience and the names of three referees, should be submitted not later than 12th October, 1979, to Dr. R.O. Owens, Department of Natural Philosophy, University of Glasgow, Glasgow G12 8QQ, from whom further particulars may be obtained.

In reply please quote Ref. No. 4508M.
3175(P)

GRANTS and SCHOLARSHIPS

THE ROYAL SOCIETY

GOVERNMENT GRANT FOR SCIENTIFIC INVESTIGATIONS

Application for grants from the third allotment of the Government Grant for Scientific Investigations for the year 1979 should be made not later than 15 November 1979 on forms of application to be obtained from the Executive Secretary of the Royal Society, 6 Carlton House Terrace, London SW1Y 5AG.

Applicants must be British subjects domiciled in the United Kingdom. Grants may be made to promote and support research in science and to assist scientific expeditions and collections; but not for personal maintenance, payment of stipends or to aid scientific publications.
3165(H)

CONFERENCES

GOLDEN JUBILEE INTERNATIONAL CONGRESS on

ESSENTIAL FATTY ACIDS AND PROSTAGLANDINS

to be held at University of Minnesota, Minneapolis, Minnesota, May 4-7, 1980. Program will include historical presentations, plenary lectures, minisymposia and panel discussions. Congress limited to 500 participants. Write for further details from:
Ruth McIntyre, Continuing Medical Education, 143 Owre Hall, University of Minnesota, 421 Delaware Street, S.E., Minneapolis, MN 55455. W353(C)

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Nature
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FELLOWSHIPS

EARTH AND PLANETARY SCIENCES

The Lamont-Doherty Geological Observatory of Columbia University invites scientists interested in any field of the earth and planetary sciences to apply for the following fellowships: two postdoctoral fellowships, each awarded for a period of one year (extendable to two years in special instances) beginning in September 1980 with a stipend of $18,500 per annum. Completed applications are to be returned by January 15, 1980.

Application forms may be obtained by writing to the Director, Lamont-Doherty Geological Observatory, Palisades, New York 10964. Award announcements will be made February 28, 1980 or shortly thereafter. W344(E)

INSTITUTE OF CANCER RESEARCH: A three year post-doctoral Fellowship is offered in the Division of Tumour Immunology, Sutton, Surrey, for an IMMUNOCHEMIST or BIOCHEMIST with recent PhD or equivalent experience to take part with Dr J.G. Hall in a grant supported project on secretory immunity. The principal duties wil relate to the isolation characterisation and quantitation of immunoglobulins and associated molecules in body fluids but involvement with tissue culture techniques and animal experimentation will be encouraged and expected. Salary in range £4,261 to £4,805 p.a. plus London Allowance of £502 p.a. Applications in duplicate with the names of two referees to the Secretary, Institute of Cancer Research, 34 Sumner Place, London, SW7 3NU, quoting ref. 300/G/14. 3150(E)

UNIVERSITY OF YORK

BIOMECHANICAL RESEARCH FELLOWSHIP

Applications are invited for this post to work with Professor J.D. Currey in the Department of Biology. The appointment is for three years from 1 November, 1979 or later, on Range 1A, and is to study the mechanical properties of whole mollusc shells.

Three copies of applications and curricula vitae, naming three referees, should be sent by 5 October 1979 to the Registrar, University of York, Heslington, York, YO1 5DD, from whom further particulars may be obtained. Please quote reference number 11/6111. 3174(E)

ST. GEORGE'S HOSPITAL MEDICAL SCHOOL London SW17 0RE

POST-DOCTORAL RESEARCH FELLOWSHIP IN BIOCHEMISTRY/ IMMUNOLOGY

Applications are invited for the above post which is principally concerned with the biochemical and immunological characterisation of the cell surface of recently established human tumour cell lines. The appointment which is supported by the Cancer Research Campaign is for three years with the possibility of extension.

Applications and further particulars to Dr D A W Grant, Lecturer in Biochemistry, or Professor J Hermon-Taylor, St. George's Hospital Medical School, London SW17 0RE (01-672 1255, Ext. 4716). 3160(E)

EARTH AND PLANETARY SCIENCES

The Lamont-Doherty Geologic
Observatory of Columbia Universi
invites scientists interested in any field
the earth and planetary sciences to app
for the following fellowships: tw
postdoctoral fellowships, each awarded f
a period of one year (extendable to tw
years in special instances) beginning
September 1980 with a stipend of $18,5
per annum. Completed applications a
to be returned by January 15, 1980.

Application forms may be obtained
writing to the Director, Lamont-Doher
Geological Observatory, Palisades, N
York 10964, Award announcements will
made February 28, 1980 or short
thereafter. W336(E)

NEW ZEALAND POSTDOCTORAL FELLOWSHIP

Experimental nuclear physics.
fellowship with a difference. Join a sm
but lively group with a 4 MV tande
accelerator and lots of beam time. O
interests are nuclear structure and we
interaction studies.

Write for information to: Prof. A.
Poletti, Department of Physics, Univers
of Auckland, Private Bag, Auckland, N
Zealand. W352(E)

UNIVERSITY OF EDINBURGH

DEPARTMENT OF PATHOLOGY

RESEARCH FELLOWSHIP

Applications are invited for a post-doctc
Research Fellowship to join a grc
studying the factors responsible for D
stability and degradation with
mammalian cells throughout the cell cy
and during programmed cell dea
Experience with mammalian or v
chromatin is desirable. The post is availa
from 1st October 1979 and is supported
the Cancer Research Campaign on
nationally agreed 1A Salary Scale fc
years.

Applications enclosing a Curricul
Vitae and the names of two referees sho
be sent to Dr A.H. Wyllie, Departmen
Pathology, University of Edinbu
Medical School, Teviot Place, Edinbu
EH8 9AG. By 28th September. Ple
quote Reference 5055. 3162(E

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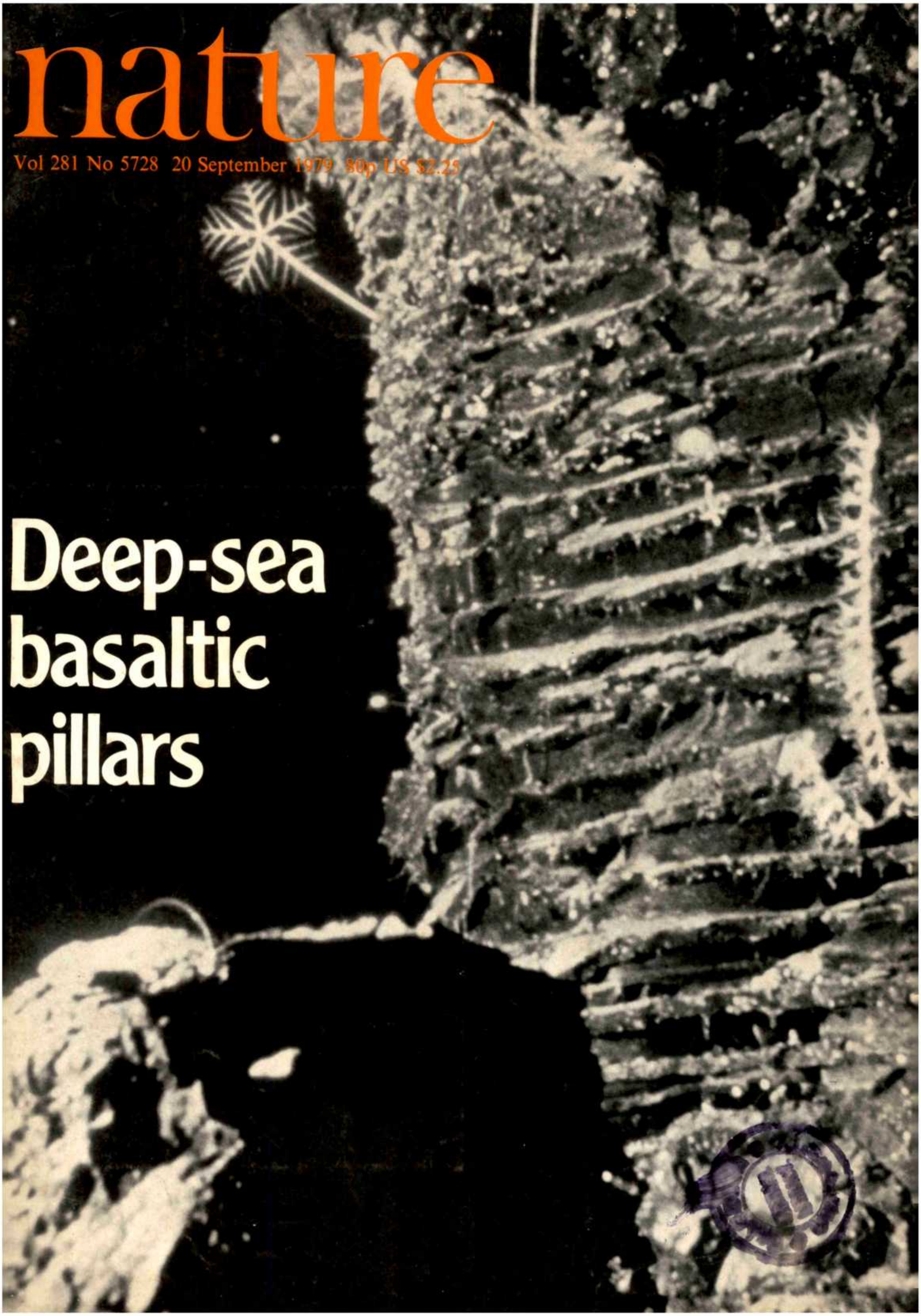

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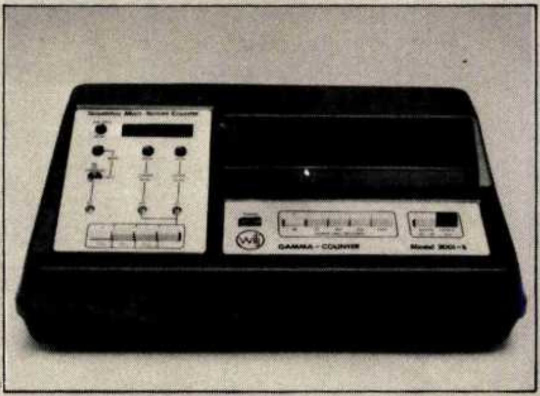

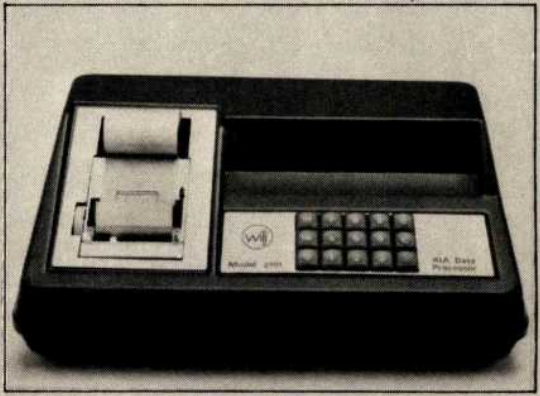

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A basaltic lava pillar at 2,600m depth on the East Pacific Rise. These novel volcanic features are discussed on pages 209–211.

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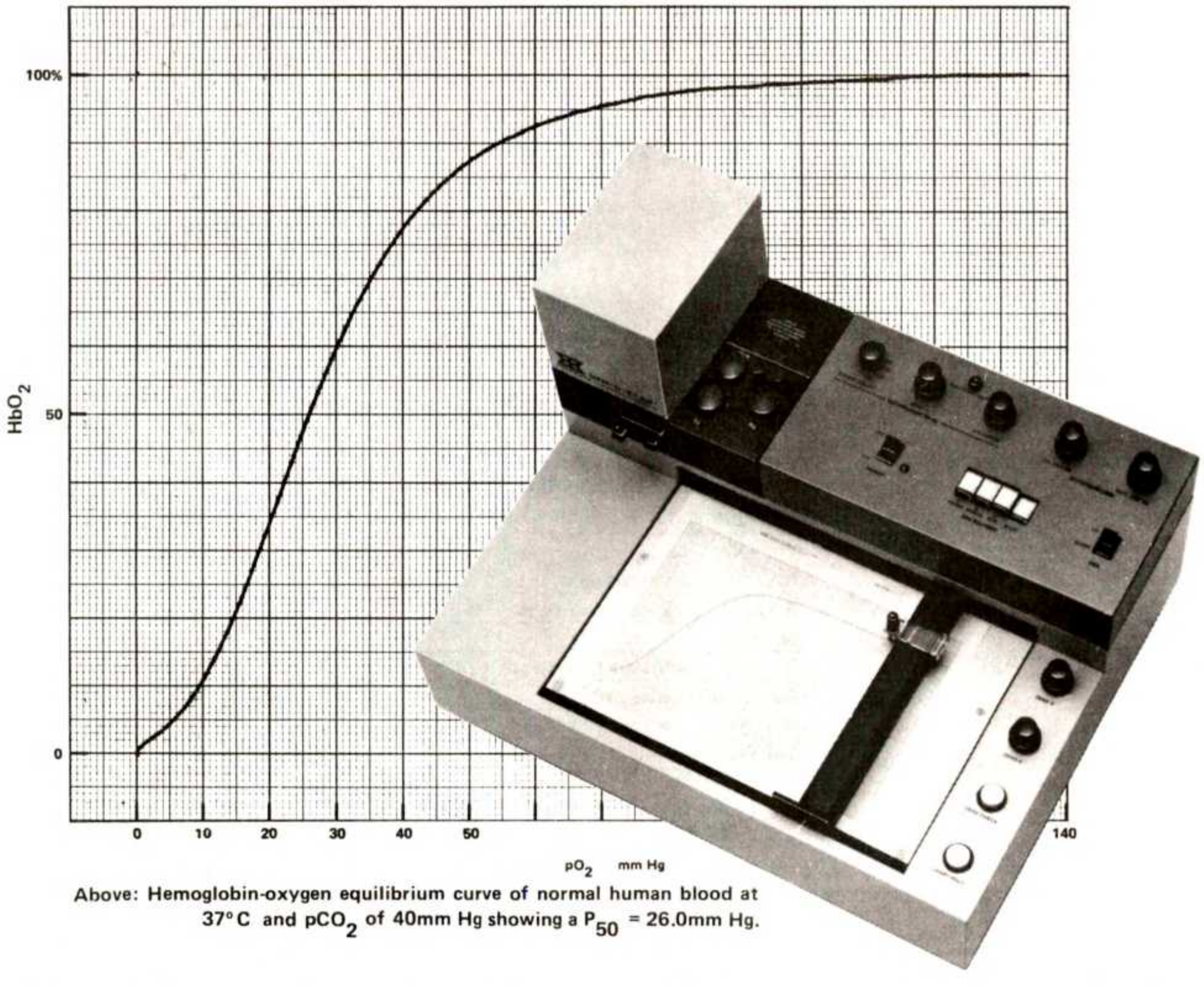

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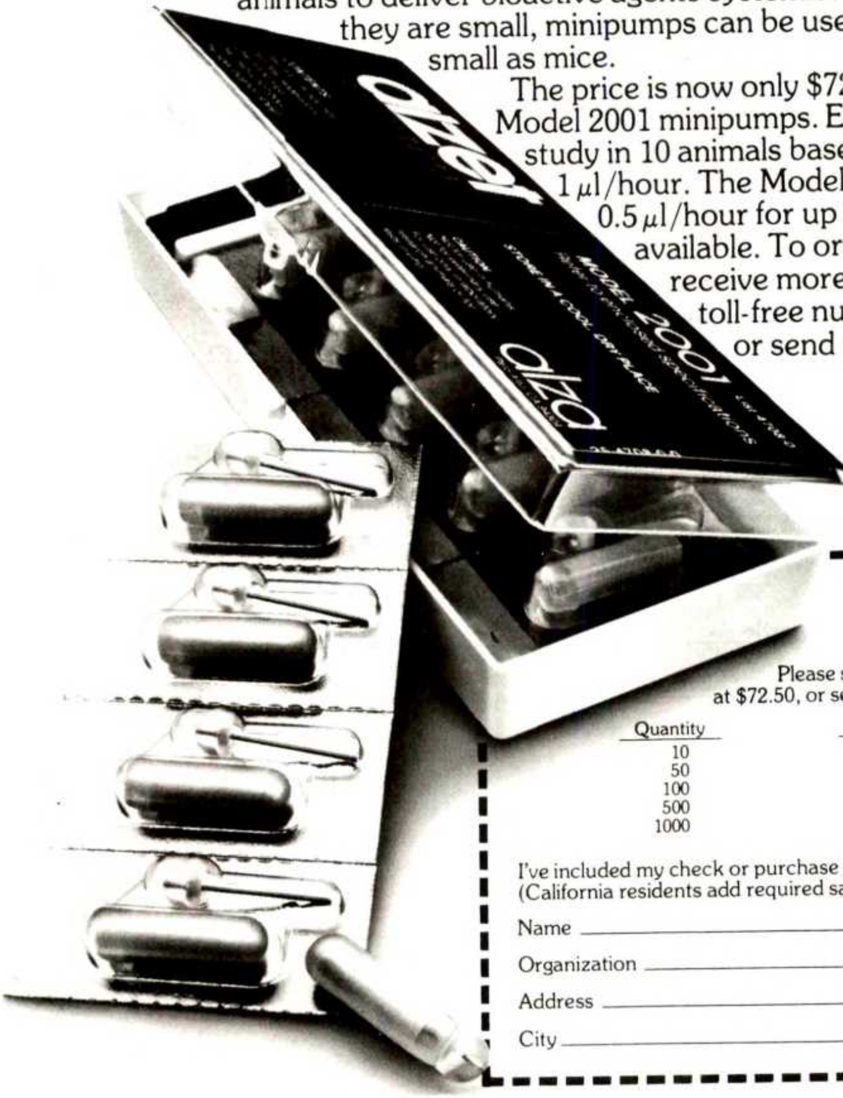

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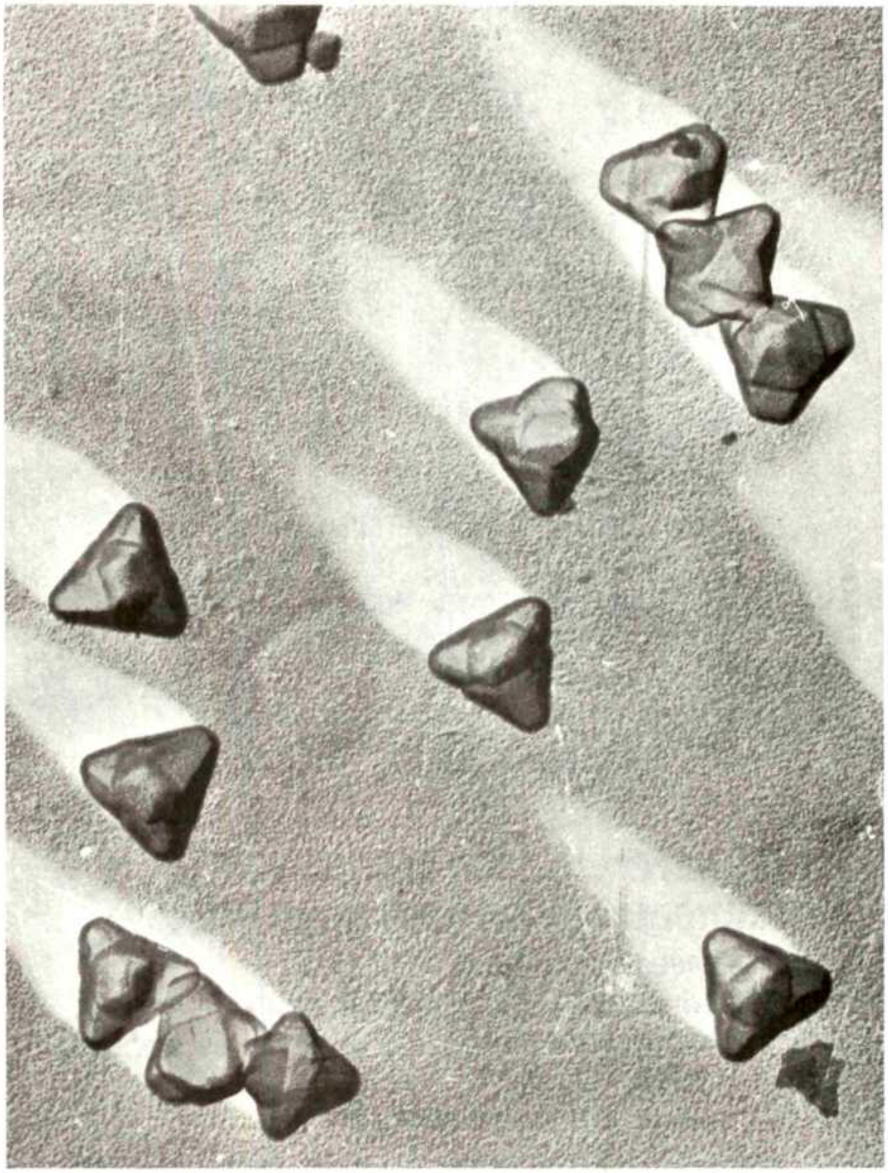

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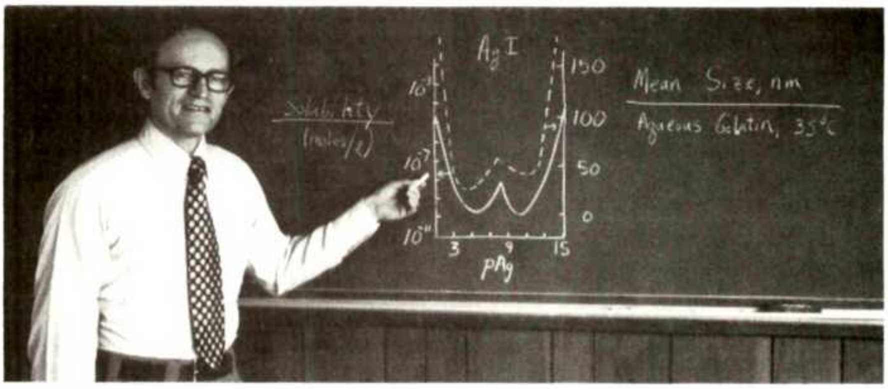

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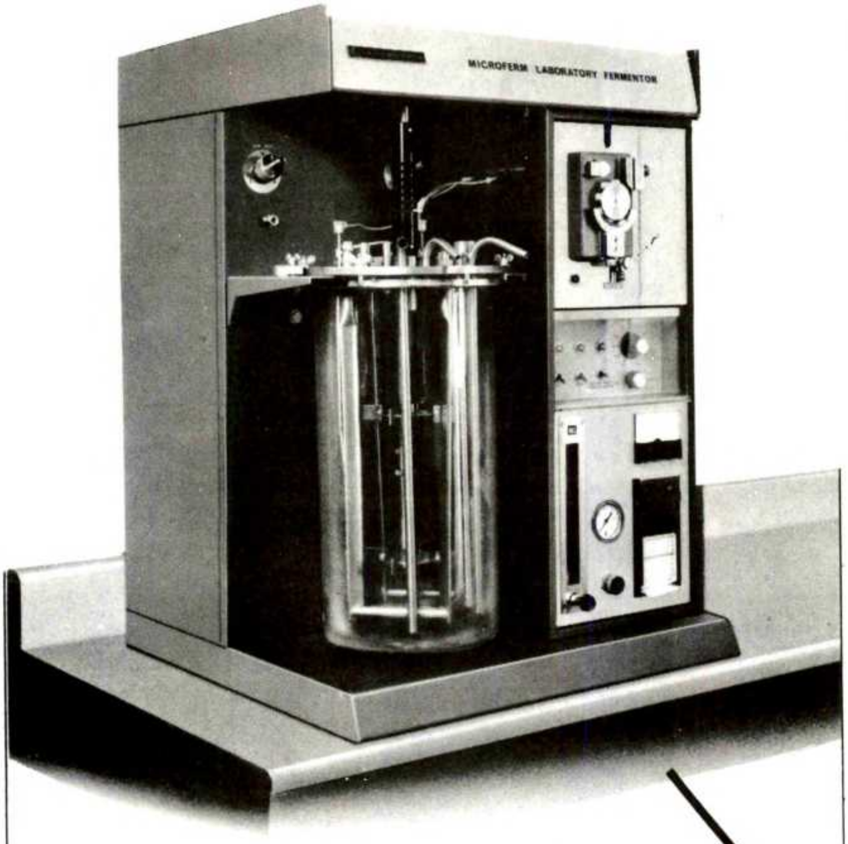

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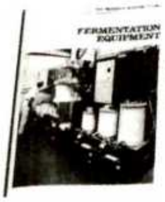

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20 September 1979

Solar energy is a key to development

Earlier this year the Commission of the European Communities held a meeting in Varese, Italy, at which scientists and technologists of both developed and developing world discussed the potential for solar energy in the latter. This meeting had been preceded by five regional seminars in Nairobi, Bamako (Mali), Amman, Caracas and New Delhi. The papers of the conference have recently been published (*Solar Energy for Development*, Martinus Nijhoff, The Hague) and raise some interesting questions concerning a science and technology whose relevance to development is very close indeed.

In the developed world, it is going to be a very long haul for solar energy to supply a significant fraction of our needs — estimates generally range around a contribution of a few per cent at most by the year 2000. This slow rate of progress is less a reflection on lack of scientific and technological skills than on the enormous built-in momentum that modern industrialised society has which strongly resists major changes of course. The picture is very different in the developing world. There solar energy (in the form of direct heating, biomass and wind) is, and always has been, the dominant energy input, particularly in rural environments. In Africa, for instance, even by 1985 not one in ten of all villages will have an electric power supply.

In many ways this dependence on the sun should be regarded as a strength for the future, which should not lightly be yielded up to the blandishments of other sources, for the poorest people of the world can ill afford to get caught in the spiral of energy price increases. And in the long run, as Sir George Porter pointed out to the meeting, if and when the technology of chemical fuels is developed, it is the barren deserts of the world which could well become the ideal place for growing these fuels — and hitherto resource-poor countries could become major energy exporters. There is also a fairly hardnosed political reason why developed countries would be encouraging developing countries to think solar — that it might just head off some nations from thinking nuclear with all the complex ramifications that would bring.

All of this clearly adds up to a compelling case for the vigorous pursuit of more research and development into uses of solar energy. Favoured as many developing countries may be in their use of cheap uninterruptible energy from the sun, they cannot develop much further without demanding more energy from somewhere.

If solar energy is to be even more widely used in the developing world, two major obstacles will have to be overcome. The first is the lack of status for the subject. Someone who designs a new solar water heater or crop dryer or desalination plant may contribute far more to development than someone who works on an exotic theoretical problem in an academic environment. And yet the latter will get more acclaim, particularly from an international community. It is no surprise that the developing world only started to show strong interest in advancing solar science and technology when developed countries (and particularly the United States) went in for it in a big way. But even now, it is not at all clear that adequate intellectual resources are being devoted to the problems.

The second obstacle is the understandable feeling in most developing countries that research and development, if done at all, has to be done domestically. Collaborative regional centres of research, where they exist at all, often get the problems that have lowest priority. But the sort of questions raised in solar energy research rarely fit snugly to national boundaries, and there is every reason to pool resources over quite wide regions. Slowly this is being realised in Africa and Latin America, but there is much scope still for more effective collaboration, if only at the level of the exchange of information.

Solar energy could be a large ingredient in the economic advancement of the developing world. It offers a marvellous opportunity for the use of indigenous skills. Will the developing world take up the challenge? □

Environmental regulations under attack

The end of a decade is inevitably a period of taking stock, looking back at the impact of past actions and forward to their implications. Ten years ago the environmental attack on the unwelcome side-effects of rapid industrialisation was at its height. Environmental protests became an increasingly common event across the western world. Demonstrators' concerns ranged from the effects of pesticides on wildlife to the carcinogenic hazards of the workplace. In the US, Congress responded with legislation such as the Natural Environmental Protection Act of 1970, and the Occupational Safety and Health Act of the same year. And the air rang with 'end of an era' quotations about how society was learning from the error of its past ways.

We may be a few months early in bringing down the curtain on the 1970s. But in recent weeks, political events in the US have begun to overtake the calendar in signalling the end to the spirit which has pervaded much of the past decade — already referred to by some as the 'golden age of environmentalism'. Bold commitments to guaranteeing a clean and safe environment, with the federal government being given a prime responsibility for achieving each, are giving way to increasing calls to pass this responsibility back to the market plce and to the private sector. The effects of regulation on energy supply and on technological innovation are usually mentioned as the most important reasons for this shift. But both are backed by a growing anti-government tendency in US politics, with Washington's role as chief regulator placed squarely in the stocks.

Over this next three weeks we shall be running a series of news features on this trend and its implications for the future. In this issue we examine (page 168) the growing conflict between environmental and energy demands. Next week we shall look at the industrial response to recent moves in the fields of occupational health and safety. Finally we shall examine how, in the international nuclear energy domain, US attempts to control the spread of nuclear technology have met with, at best, only limited success.

We do not wish to speculate too much on what on the next decade has in store. But the movements of the past decade will occupy an important chapter in the history of the relationship between science and politics. The movements of the next are unlikely to be any less significant. □

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The end of the age of Aquarius?

Concerns about energy and inflation, linked to a conservative trend in US politics, are beginning to eclipse the moral commitment to a clean and safe environment characteristic of the past decade. In the first of three articles on how this has happened and what may follow, **David Dickson** describes how the energy issue has become a major threat to US environmental movements.

"Man can survive pollution; environmentalists can relax about that. The question is whether he can survive regulation." These words headed a full-page advertisement in the US national press last week for a new book, *Restoring the American Dream*, by best-selling author Robert J Ringer.

The sentiments are not new. Indeed such arguments have been frequently heard since concerns about the effects of pesticides on bird-life ushered in the environmental movement in the mid-1960s. And they have provided a constant back-drop to a decade of legislative measures designed to protect the US environment against the worst excesses of technological growth.

In the past few months, however, concerns about growing inflation and the security of future energy supplies have both helped to generate an anti-regulation bandwagon that is demanding a major rethink of this legislation and its effects — as well as laying the ground-work for a substantially different approach to environmental regulation in the 1980s.

As far as the administration is concerned, this will mean less emphasis on the government as a regulator of industrial activity, and greater emphasis on the market-place as the matrix for 'rational' decision-making on regulatory activities.

For the environmental movement, it means a shift from the essentially defensive posture which the movement has been adopting in recent years, capitalising on the gains made earlier in the decade, to one in which urgent action is now needed in most fields (except perhaps that of nuclear energy) to prevent the threat of a complete rout.

Two main factors have fuelled this anti-regulatory tendency. The first has been the argument that inflationary problems are directly linked to a decline in national productivity (which fell by an annual equivalent rate of 5.9 per cent in the second quarter of 1979); and that this is largely linked to a decline in technological innovation, itself due partly to increased environmental and health regulation.

"What started as a regulatory snowball ten years ago has become an avalanche, and innovation is one of its victims," Dr H D Doan, ex-president of the Dow Chemical Company, said at a meeting of the American Chemical Society in Washington last week. "George Orwell may not have been accurate in his predictions; but the regulators tend to confirm his foresight."

The second factor which has played a key role is the pressure on US politicians, following the Iranian oil crisis earlier this year, to be seen to be doing something about guaranteeing future energy supplies — if necessary at the expense of environmental considerations.

Two developments in particular have led to a qualitative rise in the energy *versus* environment debate. The first has been the push for a massive programme to stimulate the production of synthetic fuels as a substitute for imported oil, and the renewed emphasis on the use of coal, both of which,if not properly handled, could have a significant impact on factors such as the build-up of atmospheric CO_2.

The second factor, which many environmentalists fear could be even more damaging, is the president's decision to establish a top-level Energy Mobilisation Board. Of particular concern are the strong powers which many congressmen would like this board to have to over-ride federal and state environmental legislation in order to speed up major energy programmes.

The idea that there are necessary trade-offs between energy demands and environmental considerations is not new. Until relatively recently, however, the administration has sought to contain such debates within a framework of cost-benefit analysis, using economic pay-off as the means of weighing up the merits of one course of action against another.

Such analysis, for example, has been behind some recent decisions to relax certain aspects of clean air legislation. For example, many power companies are being squeezed between new limits on sulphur dioxide emissions, and the president's call to reduce oil consumption by half by 1990, largely through the use of coal; and cost-benefit analysis is being used as a prime tool to tackle such conflicts.

In the past few months, however, this approach has been overtaken by a more overtly political drive aimed at circumscribing the government's role in a range of activities. One of the first manifestations of this trend was California's decision last year to halve its property taxes, the famous Proposition 13; more recently, government regulators have become a prime target for political abuse.

According to a survey published in March by Opinion Research Corporation, for example, more than half of the general public feel that the costs of government regulation exceed the benefits. And by a margin of two to one, those interviewed said that there was a need for less rather than more government regulation.

With elections coming up next year, congressmen from both parties are eagerly responding to what they see as popular demands for less regulation — in many cases outstripping the administration's economists in their anti-regulatory fervour. In the last two weeks, for example, this tendency has become clear in four separate congressional actions.

The first was an amendment by Senator Dale Bumpers (Dem. Arkansas) to a bill on the federal court system, requiring a federal agency to demonstrate the validity of a particular set of regulations in court every time that they are challenged. The amendment thus challenging the "presumption of validity" that now covers agency rules when challenged in court proceedings, making it easier for courts to overturn federal regulatory decisions.

The second decision was taken during a debate last Tuesday on a strip-mining bill, which would allow states to disregard regulations laid down by the Department of the Interior about making-good disturbed landscape. On the same day, the Senate also voted to allow the completion of the Tellico Dam, thus in effect agreeing to the extinction of the snail darter, whose status as an "endangered species" had previously led to a Supreme Court ruling that the dam should not be finished.

Perhaps the most significant decision, however, was taken by the House Commerce Committee. In discussing the role of the President's proposed Energy Mobilisation Board, the committee agreed that the board should have considerably stronger powers than the president had proposed, enabling it to waive environmental regulations.

Despite intense efforts by environmental

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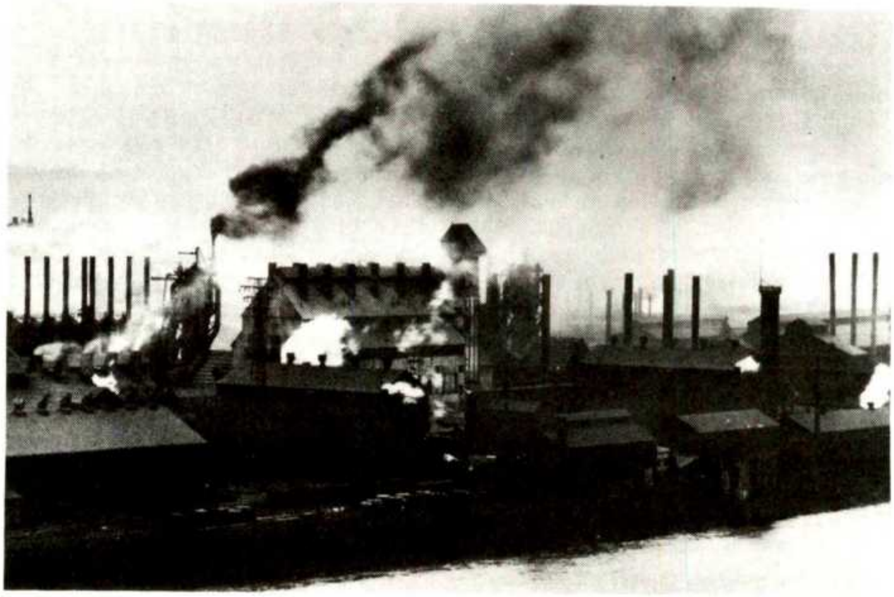


lobbying groups, the committee approved a bill which would allow the board to recommend that the president waive any federal, state or local law blocking a high-priority energy project.

Environmentalists are supporting an alternative version of the bill under which, as originally proposed by the adminstration, the board would be merely able to speed-up procedural aspects of project approval; and one sympathetic congressman, Representative Tim Wirth (Dem. Colorado), has promised a "huge, huge battle" when the Commerce committee bill is debated by the full House.

Critics of the stronger version of the bill have been particularly concerned about the apparent support that this version is now receiving from the White House (even though administration officials claim that their support is at present essentially a bargaining device).

"If we are not careful, the Energy Mobilisation Board could threaten fifteen years of environmental work," says David Maselli, energy policy director for Friends of the Earth. He points to the low priority given to environmental considerations in the energy proposals with which President Carter emerged from his recent seclusion at Camp David; "it was close to the bottom line — so close to a 'worst case' guess about the outcome of Carter's deliberations that many environmentalists were genuinenly surprised," he says.

Maselli points out that environmental groups have not lost out on everything in recent legislative debates. They have had considerable success, for example, in slowing down the projected growth of the administration's proposed programme for synthetic fuels — an issue against which "we are standing as hard as we can" says Herb Epstein of the Solar Lobby — and have won acceptance for numerous solar energy and conservation measures.

But no-one denies that there are some major battles ahead. "I think it is going to be a hellish struggle, even to preserve the gains that we have won," says Epstein. And others are already saying that the 1970s may be looked back on as "the golden age of environmentalism."

Speaking at the ACS meeting last week, Dr Frank Press, director of the Office of Science and Technology Policy, indicated a shift in administration thinking when he said that he saw a "new philosophy" being pursued. This was one that recognised that the desired goals of environmental, health and safety regulation might best be achieved through incentive-oriented, rather than a direct federal regulation, approach.

"Such an approach, rather than commanding that specific technologies be applied to achieve the specific standards, allows the industry to choose its own paths to achieving the same end results," he said. And he gave as an example the "bubble approach" which the Environmental Protection Agency had introduced to air pollution, in which a plant can set its own effluent release from each point source as long as the total effluent release complies with a federal standard set for the entire complex.

"One of the important things to recognise regarding this approach to regulation is that it provides new incentives for industry to innovate. It takes some of the repressiveness and uncertainty out of regulation, and relies more on market forces to achieve the desired goals in environmental quality, health and safety."

The need to allow an increased, if not dominant, role for market forces is also emphasised in a report *Energy: The Next Twenty Years,* sponsored by the Ford Foundation and published in Washington last week by Resources for the Future.

"Air pollution control policy should concentrate on providing incentives for making progress toward cleaner air in a way that is cost-effective over time," says the report, produced by a group of academics and industrialists. Attempts to define rigid "safe" exposure levels are both scientifically and administratively unsound, the report recommends moving towards "a different philosophy" of pollution control, deemphasising rigid deadlines and standards, in which market processes determine the details of ways to meet particular environmental goals.

The new shift is also likely to have an effect on the academic environmental science community. "The new climate for environmental regulation makes the need for accurate scientific data even more important than before; in particular there will be a need for better epidemiological data, looking at the real effects both of pollutants and of environmental regulations," says Dr John E Cantlon of Michigan State University, chairman of the National Academy of Sciences' Environmental Studies Board.

"I do not think that all the census work that I have seen indicates that people are willing to give up clean air or clean water. But they may be more willing than before to ask the question of whether the cost-effectiveness level of certain regulations is defensible," says Dr Cantlon.

Finally the environmental movement is beginning to realise that the strategies which it used so successfully in the early 1970s to achieve significant legislative victories may no longer be appropriate for the form of the debate in the 1980s. And that it may be necessary to form new coalitions — particularly with labour or other political groups — if it wants to remain effective.

"One of the weaknesses of the environmental movement is that it has never been able to deal properly with economic issues. But recent moves by the administration on issues such a sythetic fuels mean that the stakes have been raised, and we must deepen both our understanding and our political linkages if we wish to remain effective," says Gail Daneker of Environmentalists for Full Employment.

What effect environmental issues have on next year's presidential election remains to be seen. Certainly many of those who actively supported President Carter earlier in his administration are growing increasingly sceptical. "The one thing worse than having a public figure against you is having a powerful public figure who used to be on your side against you," says David Maselli, referring in particular to the administration's apparent support for a strong Energy Mobilisation Board.

But few see the issue merely in terms of personalities — or of presidential politics. Rather it is a response to an economic and political climate fundamentally different from that which saw the dawning of an "Age of Aquarius" in the late 1960s. With demands for a decreased government role in regulation, and an increased emphasis on market-place economics, moral commitments no longer carry much political capital. □

Next week: health and innovation

ICSU to raise human rights issue at 50 meetings

This summer, a new committee on the 'Safeguard of the Pursuit of Science' of the International Council of Scientific Unions delivered its first report to the ICSU general assembly. The purpose of the committee is to investigate and record cases of scientists unwarrantedly dismissed from their posts or otherwise hampered from pursuing their careers. During its first year's work, the Committee had been apprised of around a dozen such cases, in countries ranging across the whole political spectrum.

This low count, however, does not represent a sudden worldwide liberalization of hitherto oppressive regimes. Rather does it reflect the extremely unobtrusive manner in which the Committee has commenced its activities. For example, not a single case of *berufsverbot* was notified to the Committee. This does not, alas, mean that after all, there are no West German scientists suffering for their allegedly left-wing views. A few days ago, Dr Andreas Dress of Bielefeld University, a leading campaigner against *berufsverbot* in science, told *Nature* that he had not heard of the Committee.

At the 1978 General Assembly of ICSU, when the Committee's founding statement was finally approved (this is a most diplomatically worded document, incidentally, avoiding all such emotive terms as 'human rights'), it was decided that the work of the Committee was an internal affair of ICSU. Accordingly, the statement was never officially issued to the general or scientific press, and only reached *Nature* by informal means almost a year later. Information about the Committee, it was decided, should be disseminated via the usual channels — the national representatives to ICSU (normally national academies of science or their equivalents).

This inevitably suggests a Catch-22 situation. An academy which was actively or passively endorsing the kind of discrimination and repression which it is the Committee's brief to monitor, would hardly be expected to pass on information to its potential victims as to how to obtain a measure of redress. The Committee, however, is less pessimistic. Its Secretary, Professor John Humphrey of the Royal Post-Graduate Medical School, london, told *Nature* that "We start with the premise that the national representatives are no happier than we are that their scientists are being removed from academic life". He admitted, however, that one cannot assume that in such countries the scientific establishment would disseminate news of the Committee very vigorously.

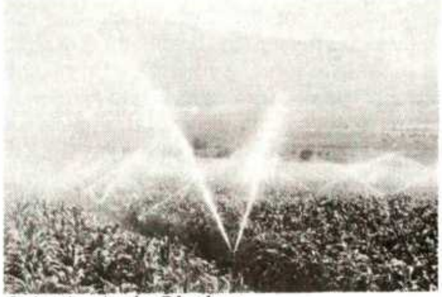
To spread the word more rapidly, therefore, the Committee has decided on a limited publicity campaign. They will display and distribute the founding statement and the forms for reporting abuses at some 50 international scientific meetings in different countries and different disciplines. Visiting scientists, who have not yet heard of the committee through their own national organizations will, it is hoped, be able to publicise the committee when they return home.

The committee's main brief is to investigate and record abuses of a scientist's right to work in his profession. ICSU is a prestigious body, and it is hoped that the moral censure it can impose will have a considerable restraining influence. In the case of highly oppressive or arbitrary regimes, where citizens are likely to disappear without trace, it has been suggested that the committee should play a more active role. Instead of waiting for cases to be reported, the committee could keep a list of all known scientists working in the country, and take as a warning signal any untoward silences or absences from conferences at which they might be expected to appear.

Moreover, in the coming year, the Committee plans to extend its activity to a more general impediment to the free pursuit of science — the vexed question of official secrecy. A working part is to meet to discuss "the losses to science that arise from all kinds of restriction on communication". A total disappearance of the concept of 'classified information' is not envisaged, Professor Humphrey stressed. Rather there would be discussions of how to remove unnecessary restrictions, whether scientists possess any special status, the significance of boycott to the growth of science and so on. It would be, he added "a Pugwash kind of thing, dealing with the impact of restriction of communication of world science, rather than with the rights of scientists."

Vera Rich

Bulgaria — proud of science on the farm



Irrigation in the Rhodope

"If we say that land is unproductive, it is we who are to blame. It simply means that we have not yet discovered the most rational type of crops for it". So Professor Milko Iordanov, a leading Bulgarian agronomist, told journalists recently. Appropriately, he was speaking at one of the show-pieces of Bulgaria's latest development in agricultural planning and research, the "Georgi Dimitrov agro-industrial complex", in the Rhodope foot-hills.

Agro-industrial complexes, explained Professor Iordanov, are essentially a further stage in the process of amalgamation which, "from 9 September, 1945" onwards, led to the formation of large collectives and cooperatives out of peasant small-holdings. By 1970, he said, it was clear that even cooperatives of 6000 to 12,000 ha still did not permit the "optimal use" of modern technology. Accordingly, the Party adopted as a special aim, the creation of agro-industrial complexes, in which several villages are combined into a single production unit, with its own food-processing plants and agricultural research institutes. Within each complex, said Professor Iordanov, each farm still retains considerable autonomy. The complex, however, is the "general leader", in charge of capital investment and overall policy. Typically, each complex will produce a wide range of crops. At the "George Dimitrov", these change with altitude from orchard fruit, through grapes, tomatoes, hazel nuts and almonds, to potatoes, and finally, sheep grazing.

The research institutes established, in Professor Iordanov's words "for complexes working on a large scale" are subsidised by national funds. Their results and experience are passed on free of charge to any other complexes which are interested although, should experts as well as information be required, it appears that some kind of bilateral contract is established between the complexes, National funding, Professor Iordanov intimated, is given "according to progress", and the research plans are in a considerable extent tied in to the national economic plans. Thus, at the "Georgi Dimitrov", work is going forward not only on specific agricultural problems but also the more general themes of saving energy and material resources. Already, said Professor Iordanov, they have succeeded, by combining a modified American "tilter" cultivator and a Soviet tractor, to obtain the same productivity with a considerable saving of metal.

For the most part, however, research is concentrated on crop investment and the selection of the most productive strains. There is considerable emphasis on "technologies for abroad". Libya already has orchards grown from Bulgarian seeds, and is sending back fruit under a compensation deal. Within Comecon, which has a common research programme on crop selection, the Bulgarian agro-industrial complexes likewise play a major role — indeed, Professor Iordanov admitted — he himself happens to be coordinator for the whole programme.

Vera Rich

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news in brief

CIA told to reveal university research links: The US Central Intelligence Agency is fighting a court order to reveal the names of universities at which it supported research into the effects of drugs on human behaviour during the late 1960s, under a programme code-named M K-ULTRA. A Washington judge has given the agency until the beginning of next month to disclose the names of the universities involved. Judge Louis Oberdorfer gave this ruling following a Freedom of Information Act suit which had been filed by John Cary Sims and Dr Sidney M Wolfe of the Health Research Group. However at the same time, the judge also suggested that the CIA might be able to argue that releasing the names of the universities would involve a "clearly unwarranted invasion of privacy" of the individual research workers. He also said that the names of the universities themselves might be reclassified as information which it was "in the interest of national defense or foreign policy" not to reveal. John Sims of HRG said last week that the names of a number of the universities involved in the experiments were already known, but that it is important to establish the basic principle. "Information about the MK-ULTRA programme raises the question of what right students and staff have to know about the research being conducted on their campus. Furthermore institutions which have already been identified as working with the CIA have been forced to face publicly the issue of what their relationship with the agency should be."

A spokesman for the agency said last week that it would attempt to persuade the judge that it was still entitled to withhold the names of the universities involved in the programme. It is understood that the director of the CIA, Admiral Stansfield Turner, has already written to all the universities concerned asking if they object to having their names revealed; and that some have replied that they would prefer their involvement with the agency not to become public knowledge.

Thibault named head of reorganised CNRS: Professor Charles Thibault, 60, has been appointed to the newly created post of president of France's reorganised Centre National de la Recherche Scientifique. Thibault, a specialist in reproductive physiology is director of research of INRA, the French agronomy research institute, and has held the chair in the physiology of reproduction at the University of Paris since 1967. He is known for his work on *in vitro* mammalian fertilisation and is a firm advocate of research oriented towards applications. His appointment is expected to accelerate the government's controversial drive to place French research on a more commercially profitable basis.

6000 demonstrate against Scottish nuclear power: SCRAM, the Scottish Campaign to Resist the Atomic Menace, a coalition of 40 anti-nuclear groups, organised one of Edinburgh's largest demonstrations last Saturday as part of a campaign against the development of nuclear power in Scotland. Speakers outlined the dangers of nuclear power generation at each step of the process from mining to waste disposal. Uranium mining, scheduled for Orkney, was singled out for special emphasis because of the large scale environmental disruption that is known to be caused by a process requiring tons of ore to be mined to produce ounces of uranium. In addition the hazards of radon releases associated with mining and toxic chemical wastes associated with the extraction process were emphasised.

Other speakers drew attention to recent controversy surrounding the hazards of low level radiation expected to be associated with the operation of the projected plant at Torness and the drilling for prospective waste disposal sites in northwest Scotland and Ireland was strongly opposed. Local feeling against nuclear dumping runs high and one speaker drew loud applause with the comment: "If it's so safe, why don't they do their drilling in London?" The demonstration attracted trade union representation for the first time in the UK. Mr Mick McGahey, president of the Scottish miners told the rally that nuclear power should not be developed until a comprehensive energy policy is evolved and until strict safety standards could be guaranteed. Coverage of the demonstration was enlivened by the presence of actress Julie Christie (below left) who marched with the Parents Against Tornes group. Christie is opposed to the 15 waste disposal drilling sites, one of which is scheduled for Powys, Wales, where she lives.

"I said commercial — not commercials!"

ESA moves to commercialise Ariane space launcher: The European Space Agency is putting the finishing touches to a French plan to create an industry-controlled corporation to administer Ariane, the European space launcher. Tentatively called 'Ariane-Espace' the new organisation will have control over manufacture and launching of all future spacecraft with government represented only by a minority control. Raymond Orye, head of the Ariane programme explains the move in terms of the usual industry-government relationship. "Once a product is developed it should be handed over to industry for use" says Orye. On the question of industrial control of the corporation Orye explains that the capital risks involved means that "industry needs liberty in order to get maximum return on their investment."

The commercial applications are largely in telecommunications, with 75% of future missions having telecommunications applications. But other exploitable areas are expected to be in Earth resources surveying and in meteorology. At present ESA is in advance of the US in turning over the results of space research to private development. The 11 member states are in broad agreement on the plan and the details on contractual and legal matters, price policy and launch control are being debated in a series of closed Council meetings. The next meeting is scheduled for 10-12 October in Paris.

Traditional Chinese medicine takes new direction: Following a recent document issued by the Party Central Committee on applying the skill and experience of experts, the Beijing (Peking) Health Department has organised its research work in traditional medicines on a new basis. Thirty nine experts have been chosen to take on apprentices or assistants for clinical practice to learn particular skills in treating certain diseases.

In the meantime new books on the clinical practices of individual experts in traditional medicines have been compiled by a team of 40 experts working over the past four years. These include *Liu Fengwu's Experience in Gynaecology, Selection of the Medical Experience of Guan Yubo* (an expert in hepatitis) and *A Collection of Clinical Experience of Zhao Bingnan* (a noted dermatologist) with books on surgery and paediatrics to be issued soon. Work of this sort was started in 1959 but was interrupted by the Cultural Revolution when many academic records were lost and some of the experts were persecuted badly. In 1975, the work was renewed at the bidding of Premier Zhou Enlai.

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Asbestos: killer dust, says book

A new book on asbestos claims half a million will die in the UK of asbestos-induced cancer. **Alastair Hay** reports

Five hundred thousand people will die from asbestos-related diseases in the UK in the next forty years according to a book published recently by the British Society for Social Responsibility in Science. In 'Asbestos Killer Dust' the author, Dr Alan Dalton, says that a 1978 US Government Report on asbestos-related cancer deaths makes possible the prediction that a "real epidemic of asbestos related diseases" is about to occur in Britain.

The US report, published by the National Institute of Environmental Health Sciences (NIEHS) estimated that over 2 million American workers will die of cancer as a result of occupational exposure to asbestos. Thus, says Dalton, in Britain — with a population roughly a quarter of that in the US — it can be assumed that asbestos will kill some half million people.

Is the future that grim? It could be, says Dalton, if the recent reports from the US are correct and if Britain does nothing about the current standards regulating asbestos fibre concentrations in the workplace. The standards — based on the number of fibres per millilitre of inhaled air—differ for the various types of asbestos used. Current UK recommendations are 0.2 fibres ml^{-1} for blue (crocidolite) asbestos and 2.0 fibres ml^{-1} for white (chrysotile), brown (amosite) and any other fibre.

According to Dalton the present British standards allow much too high a concentration, and far from being safe present a positive danger to health. Citing a 1976 US National Institute for Occupational Safety and Health (NIOSH) report that there is *no* safe level for asbestos, the author questions the justification for the British standard.

Dalton points out that, in its report, NIOSH recommended a new US standard of 0.1 fibre ml^{-1}—but that even this was chosen because of practical problems in taking accurate measurements of asbestos fibre concentrations. Why then, the author asks, is the British standard for white asbestos 20 times that recommended for the US? And why he questions, does Britain persist with its two-tier standard for blue and white asbestos when NIOSH recommends one standard for both? Mesothelioma, the asbestos-related cancer, Dalton points out, is now quite clearly attributable to exposure to both types of asbestos and not just to the blue form as was originally believed.

The British standards are currently being reviewed by the Advisory Committee on Asbestos of the Health and Safety Executive (HSE), and a report stating the Executive's view on the adequacy of the standard is expected in the next 2-3 months. It has been reported that the committee will recommend that the standard for white asbestos should be halved to 1 fibre ml^{-1}, matching Sweden's limits; that the limit for brown asbestos should be reduced to 0.5 fibre ml^{-1}; and that blue asbestos should be banned, with the standard for its removal remaining at the present 0.2 fibres ml^{-1}.

It is quite clear, however, that the original study at the Turner and Newall factory in Rochdale in 1966 which laid the basis for the British standard was flawed. The number of asbestos-related health problems was considerably underestimated. Only workers who had completed 10 years service and were still in the company's employ were included in the study; those who had left the factory were not followed up. In addition the clinical test used to assess the effect of accumulated asbestos exposure has been criticised as being too stringent.

The test measures the prevalence of crepitations (abnormal sounds in the lung during exhalation and inhalation) and it has been suggested that this test might only identify more serious asbestos injury and miss cases with minor damage. Furthermore, there are indications that the dose/response data from the Turner study may in fact be wrong, as the dust level was actually higher than originally reported.

Although there is no official comment from UK Regulatory Authorities on the 1978 NIEHS report for asbestos related cancer deaths, criticisms have been made about it in private. For example, it is known that the HSE feels that figures for deaths in the US cannot be applied to the UK. Scientists at the HSE are known to have doubts about the validity of the assumption in the NIEHS report that the "relative risk" of developing cancer is constant throughout life. Furthermore assuming the most conservative estimate of risk in the NIEHS report it can be calculated that at least 5000 deaths from mesothelioma should be occurring annually. In 1976, however, the US figure was 1000 deaths.

Even though the HSE feel that Dalton has considerably over-estimated the dangers, the Executive does admit that mesothelioma deaths present a real problem. Figures collected by HSE's Employment Medical Advisory Service show that deaths from mesothelioma have risen inexorably since 1968. EMAS figures for 1968, 1972, 1974, 1977 are 154, 208, 230 and 323 deaths respectively. An HSE spokesman pointed out that the mesotheliomas may not have been due to asbestos; but he did confess that it was almost certain that they were. □

'Asbestos Killer Dust' by Alan J.P. Dalton. Published by the British Society for Social Responsibility in Science. 9 Poland Street, London W1V 3DG. Price £2.25

Desai's difficulties with AT

Alternative technology is no simple panacea for India writes **Anil Agarwal**

WITH the emergence of the Janata government in 1977, considerable hopes were raised both within the country and outside, that India would become a major Third World country actively using and supporting appropriate technology (AT). The need for AT was mentioned in the election manifesto of the Janata Party and, in his very first broadcast to the nation, Prime Minister Morarji Desai, had emphasised that "the big city, the big machine and big science have their place. But they cannot claim a prescriptive right to preference and dominance. We have to make the village the centre of our economic progress."

The industrial policy announced by the Janata government in December 1977 also recognised the importance of reducing unemployment and rural-urban disparities in India's future industrialisation programmes. Although India is today the tenth largest industrial producer in the world, the total number of its unemployed is almost equal to the total number of those employed by all its organised mills, factories and services. Mr George Fernandes, the Janata Industries Minister, therefore, declared that AT "will henceforth be an integral part of policy and government will ensure that this important area gets adequate attention." The Janata government, he stated, "will not favour large scale industry merely for demonstration of sophisticated skills or as monuments of foreign technology. The main thrust of the new industrial policy will be on effective promotion of cottage and small industries widely dispersed in rural areas and small towns."

AT, thus, appeared to be in early 1978 not only a sensible option for India, but also one of enjoying great support. However, over the months the initial resolve of the Janata government in favour of small-scale labour-intensive rural-oriented AT was weakened. This was, to a great extent, because of the myriad inter-party factional tensions, which virtually sapped the very will of the government to govern, but partly also because of the strong opposition from scientific and industrial establishments.

The Janata government's advocacy of AT was unfortunately accompanied by a barrage of criticism of the country's past scientific and technological activity. The critics argued that the country had not adequately benefited from its large investments in R&D institutions and that

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Spinning polyester: more jobs, but does it increase dependence?

the direction of past R&D activity was too urban and big industry-oriented. Eighty per cent of Indians, they argued, live in the villages yet very few technologies have been developed to meet their needs.

This criticism obviously drew counter-criticism. Professor M.G.K. Menon, the former Chairman of the Electronics Commission and the current Director-General of the Council of Scientific and Industrial Research (CSIR), for instance, strongly argued that although research can be done to find new ways of using agricultural and animals wastes in rural areas, and so on, it is nonetheless wrong to criticise the CSIR and its institutions for neglecting this kind of research because it was not in their initial charter to do so. Secondly, some scientists even argued that the government's advocacy of rural development, appropriate technology — and, in fact, even its efforts to reorganise bodies like the CSIR (See *Nature*, vol) — betrayed an "anti-science and anti-technology belief". Professor A.K.N. Reddy of the Indian Institute of Science at Bangalore, a well-known advocate of AT, alleged in an article in an Indian economic journal that there is a "widespread suspicion that the present government, in its over-riding concern for rural problems, believes that these problems can be solved *without* science and technology." Dr Reddy stated his own belief that the generation of AT is "rarely a trivial affair — it invariably requires subtle analysis, innovative experimentation and design, and sophisticated scientific and engineering thinking." According to Reddy, developing countries interested in AT must develop their own indigenous sophisticated scientific skills and that they must conduct basic as well as applied research. To take one instance, basic studies of methanogenic bacteria can greatly contribute to the development of biogas systems.

Some senior Indian scientists and science policy experts also denounced AT as a concept that is largely being promoted by those western lobbies who want to belittle India's past efforts towards technological self-reliance and keep Third World countries permanently dependent and backward. In what was an obvious reference to developments in India, Mr Ashok Parthasarathi, the current Secretary to the Electronics Commission and former special assistant on science and technology to Mrs Gandhi, told a seminar on the UN Conference on Science and Technology for Development (UNCSTD) held in Sweden in late 1978, that there is a strong body of opinion amongst the "nationalist segment in most Third World countries" that regards AT "as a diversionary tactic on the part of the North — diversionary in the sense that it seeks to redirect a significant part of the scientific and technological (S&T) capacity of Third World countries towards the development of these new 'rural-oriented', 'appropriate' technologies, leaving the main muscle of industrial power — the petrochemical complexes, the steel plants, the railways, the power plants — in the hands of transnational companies."

Indian scientists who share this argument point out that even if a Third World country were to make rural modernisation and agricultural development the prime goals of its development strategy, it would still need several sophisticated industrial products like cement, chemical fertilisers, pesticides, iron and steel, trucks, railway systems and electricity to make its strategy work. The technology required to produce all these products is today dominated by western multinational corporations.

The critics further challenge the value of diverting large scientific efforts towards the development of rural-oriented AT by pointing out the strong evidence that has emerged in recent years that what is known as AT, in a highly stratified society like that of India, tends to benefit the rural rich and not the rural poor. A prime AT like the development of biogas plants has been found to have benefitted only those landowners who possess the money and cattle to buy and operate such plants. Rural modernisation and eradication of poverty, the critics argue, require a political will for structural transformation within society. So, it is claimed, if a Third World country like India were to pay too much attention to AT it would not only fail to improve the lot of its rural poor but also, in the process, become more dependent for its modern technological needs on foreign multinational corporations.

Indian supporters of AT like Mr M.M. Hoda of the Appropriate Technology Development Association in Lucknow protest that the proponents of modern western technologies, while raising fears of a strengthening of the rural elite, are conveniently forgetting that their own technological strategy will lead to the strengthening of an already powerful urban-industrial elite. And that this elite will equally oppose any structural transformation that may be required in the long run to eradicate poverty. The argument that the Janata leaders are "anti-science" appears to reflect the elitist character of the scientific community itself. It is indeed not rare to hear an Indian scientist calling the Janata leaders a pack of 'desi' (native) people, largely because they do not possess the finesse that earlier leaders like Mr Nehru and Mrs Gandhi acquired from their western educations.

AT-protagonist Reddy, however, does criticise western organisations and aid-agencies for their excessive advocacy of AT. By giving the impression that AT is something mainly suitable for the Third World and by doing precious little to develop 'appropriate' technologies for use in developed countries themselves, Reddy argues that western organisations are not just being extremely patronising but are also producing very counter-productive results with their advocacy. Their enthusiasm, he claims, has created such a strong backlash to the concept of AT in India that even Indians are now finding it difficult to advocate it.

"What India needs is both large and small-scale technologies," explained an anti-AT senior policy-maker to me, a few months before Mr Desai's government fell. "We should not be asking organisations like CSIR to redirect their efforts towards rural-oriented AT but instead be asking them to step up their research efforts to develop both small-scale rural-oriented technologies as well as those technologies that are required by modern large-scale industry." "I think," he proudly claimed, "that we have now succeeded in educating the new Janata leaders, including the Prime Minister."

That a Third World country like India needs a mix of technologies was pointed out even in the Indian national paper to UNCSTD. The paper explains that while the broad criteria for the appropriateness of technologies must be defined by a nation's development objectives (like meeting basic needs, employment-generation, self-reliance and environmental protection), the implied productivity *versus* employment problems can be resolved only by adopting a balanced mix of capital-intensive and labour-intensive technologies, of centralised and decentralised production, and of large-scale and small-scale units.

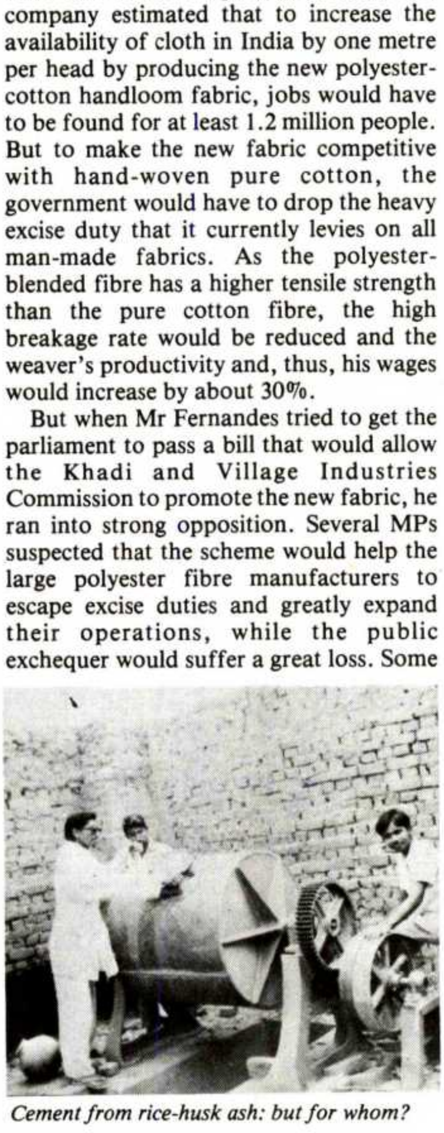
But what exactly is it that the Janata government has tried to do in the home of AT, apart of course from speeches by Prime Minister Desai and other Janata leaders that Indian scientific research needs a different direction? The clear answer is: 'not very much'. The main attempt to go small and introduce the concept of appropriateness in technology choice was restricted to the — albeit important —industrial sector, and here too the Janata government merely tried to put more teeth into a policy that all former Indian governments have advocated since the mid-1950s to protect and promote small-scale industry. The Ministry of Industries increased the number of items exclusively reserved for production in the small-scale sector from 180 — under Mrs Gandhi's government — to over 500. And to help promote the development of small industries in neglected backward areas, it launched a scheme to set up an industry centre in each district of the country. The centres will provide small entrepreneurs with all the services and support they require under one roof, including economic investigations of a district's raw material and other resources, supply of machinery and equipment, provision of raw materials, arrangement of credit facilities, quality control and research. The scheme, which started in May 1978, has had teething difficulties, but 223 centres had been sanctioned by end of 1978.

In certain industries like cement, matchsticks, soap and textile manufacture, which can be operated on a small scale, Mr Fernandes — the Minister for Industry — strongly supported efforts to make small-scale ventures succeed. For instance, his ministry provided a 50% rebate in excise duty for cement produced from mini-cement plants (with a capacity less than 200 tonnes per day). Dr S. Chopra, Director-General of the Cement Research Institute, which has developed 20 tonne per day capacity plants, estimates that mini-cement plants should be producing about 10% of India's cement within the next five years. Mr Fernandes also strongly advocated the manufacture of cement from rice husk ash, though some scientists feel this was premature, as there are very few such plants in existence in India and the quality of their product still needs to be standardised.

Under Mr Fernandes, the Ministry of Industry also asked those large industries, many of which are subsidiaries of western multinationals, which produce such mass consumer goods as soap and matchsticks, to phase out production within three years and allot it to continue in labour-intensive cottage and small-scale units. The Minister pointed out that nearly 70% of India's matchsticks were being made by cottage industries, which employed 450,000 people. But the Western India Match Company, a subsidiary of a Swedish company, which makes the remaining 30%, was employing only 15,000 workers. Mr Charan Singh, who was Finance Minister in Mr Desai's government and is a fervent advocate of rural cottage industries, tried to force WIMCO out of the market by increasing excise duties on all machine-made matches and halving that on hand-made matches.

In the textile industry too, the Janata government pushed the existing policy to support handlooms further, by forbidding further expansion in the weaving capacity of textile mills. About ten million people are already employed in the handloom textile sector, which makes it the largest employer in India outside agriculture. But unfortunately most of them are only part-time workers and their wages are abysmal. Mr Fernandes tried to introduce a new technology to upgrade this industry and thus increase the productivity of handloom weavers and their wages. But he met with strong resistance from his own party. The Industry Minister tried to promote a technological breakthrough that allowed cotton-blended man-made fibres to be both hand-spun and hand-woven for the first time. The breakthrough had been achieved by a private Indian company which manufactures polyester fibres. The company estimated that to increase the availability of cloth in India by one metre per head by producing the new polyester-cotton handloom fabric, jobs would have to be found for at least 1.2 million people. But to make the new fabric competitive with hand-woven pure cotton, the government would have to drop the heavy excise duty that it currently levies on all man-made fabrics. As the polyester-blended fibre has a higher tensile strength than the pure cotton fibre, the high breakage rate would be reduced and the weaver's productivity and, thus, his wages would increase by about 30%.

But when Mr Fernandes tried to get the parliament to pass a bill that would allow the Khadi and Village Industries Commission to promote the new fabric, he ran into strong opposition. Several MPs suspected that the scheme would help the large polyester fibre manufacturers to escape excise duties and greatly expand their operations, while the public exchequer would suffer a great loss. Some people also feared that the new fabric could drive those traditional weavers out of jobs who were not able to organise themselves sufficiently to benefit from the new technology. The new fabric could result in more unemployment. Mr Fernandes failed to get parliament's support.



Cement from rice-husk ash: but for whom?

However, an official in the Planning Commission who has long been a lone advocate of AT in official circles, pointed out that cement, for instance, even if it is produced on a mini-cement plant, will remain an inappropriate product for building houses in India as it is beyond the purchasing power of the majority of the country's population. Only a policy that promotes housing technologies which reduce the use of cement and increase the use of traditional building materials such as mud can be described as a policy for AT in the housing sector. But big ostentatious houses, built with a wasteful use of cement, are the order of the day amongst the Indian elite and such an approach to AT would require a very strong political will, something that the Janata government failed to display.

Meanwhile, however, Indian planners are certainly learning much to their chagrin that domestic technological capability can be a double-edged weapon. It can be used to subvert national objectives, just as much it can be used to serve them. The Planning Commission in 1977 had refused the State Government of Punjab permission to import combine harvesters. It had argued against these machines on the grounds of massive unemployment within the country. But the Punjab had created such a huge demand for labour that farmers within the region face crippling shortages of labour, particularly during periods of peak labour requirements. After the central government turned it down, the Punjab government approached the Punjab Tractors Limited (PTL) in Chandigarh, which it had helped to set up, to manufacture these combine harvesters. This company had been set up by an extremely enterprising group of former R&D engineers from the Central Mechanical Engineering Research Institute (a unit of the CSIR) to manufacture a small tractor that they themselves had designed. Production of the tractor started after considerable delay and opposition from those interest groups which wanted to collaborate with foreign companies. The efforts of this group for technological self-reliance have thus been widely praised and discussed. But now the PTL management cannot refuse to design and develop combine harvesters for the government of the state in which it is based even though it may not be happy to do so. The first prototype of an indigenously designed combine harvester is expected to be ready soon. Domestic technological capability thus would have served the Punjab government's purpose but subverted the Planning Commission's objectives. Now how does one control such a situation? □

UNCSTD: now we need action

Arnoldo K Ventura, Technical Director of the Scientific Research Council of Jamaica, counts UNCSTD 'a stitch in time'

After five preparatory conferences, several national, regional, and specialised meetings, UNCSTD begrudgingly approved a programme intended to tackle worldwide underdevelopment. But, because of the complexities of over-development and under-development, and the fact that the precise relationship of science and technology to development is still being unraveled, the conference was anchored on presumptions based on superficial observations and good intentions. Consequently, the resulting programme remains essentially a series of imprecise generalities which now beg for more precision and implementation.

The *ad hoc* mixture of politicians, diplomats, bureaucrats, and a few scientists, many of whom were attending the 'circuit' for the first time, or who were vaguely familiar with the nuances of the Conference, did not help the UNCSTD deliberations, while many of the few oldtimers in the UNCSTD extravaganza had already formed hardened opinions. Consequently, in spite of the fact that no legal codes were being considered, the debates were often long and tedious.

The debates lacked specificity, and it was clear that neither the developed nor the developing countries had a firm grasp on the systems, steps, or components of science and technology which are necessary to redress the problems of mal-development. Very little note was taken of the fact that while many studies have shown a strong relationship between rising industrialisation and the ferment of scientific technology, few categorically outlined comprehensive strategies for the resolution of underdevelopment based on the unique experiences of Europe, US, Japan or recently industrialised states.

As a matter of fact, the pivotal components of the science and technology process which have lead to the successes of the developed countries are still being determined. Consequently, the UNCSTD debate was replete with emotion and defensive rhetoric, rather than with compromise flowing from a framework of scientific facts or well-documented experiences. The "programme of action" used as the base document for negotiations was not action-oriented, as a result of the paucity of concrete information regarding the proper way to deploy science and technology to promote a balanced development within under-developed countries.

Although it is generally accepted that a decent level of indigenous technological competence is vitally necessary for this process to take place, the basic infrastructure necessary to provide this capacity in light of different concepts of development and differing national resource endowments, is still not fully understood. Furthermore with limited technological capacities in many developing countries and with many countries still not sure of the type of investment or sacrifice needed to attain increased capabilities, the developed countries during UNCSTD tended to view the problem of underdevelopment in terms of what can, instead of what should, be done.

The ill-effects of some modern technologies on society, on culture, and on the environment have further confused the question of science and technology for development, thereby raising doubts about the transfer of the most modern technology for the amelioration of poverty. This type of confusion of the issues often lead to extreme positions, especially among delegates exposed to them for the first time. This engendered superfluous verbiage and a train of stalling manoeuvres. Nevertheless, in general, the debates have served to emphasise the fundamental nature of the problem and may bring solutions nearer.

Against this background, it was unfortunate tht some specialised agencies decided unabashedly to use UNCSTD as an opportunity to expand their power base. This distraction from the main thrust of the Conference underscores the dilemma of poor countries, as often institutional politics looms to obliterate the pressing need to harmonize against the ravages of underdevelopment. This suggests the need for closer examination of the UN agencies and their contribution to the relief of human suffering.

Although much can be said about the imperfections of UNCSTD, its signal importance should not be overlooked. Apart from generating an increased awareness of the indispensability of science and technology to development among the developing world — the elaborate preparatory process for the Conference achieved that — the event itself brought together national decision makers and functionaries from the scientific communities to discuss concepts which are of vital interest to both. Such ventilation of the issues from different perspectives very seldom takes place at the local or national levels, and hopefully this may be the beginning of operational links between international political and the scientific circles, which may provide a clearer picture of the problems and bring us closer to collective solutions. UNCSTD gave the opportunity also for socially concerned scientists, from countries at different stages of development, to share experiences.

It is interesting to see how the voluntary contribution of some $250 million for 1980 and 1981, earmarked for the promotion of science and technology, will be utilised in the underdeveloped countries, granted the inadequate technological capacities in these areas, and the fact that the amount is paltry compared to the magnitude of the world's problems. It is clear that the major contributors will be tempted to control the expenditure of these funds, either to ensure that these allocations are used to establish a viable scientific and technological infrastructure or surreptitiously to ensure that these funds are not used to create undue competition for their local industries. It will be interesting to see how Third World scientists and technologists respond to the disbursement of these resources. UNCSTD, undoubtedly, will force some reaction from the scientific and technological communities of the Third World. Their response in turn will surely condition the attitude of their counterparts in the industrialised states in future.

There is also some hope for change — as the Conference has engendered more solidarity within the Group of 77, while there appear to be more areas of contradiction and differences arising among the ranks of the rich as they struggle to contain the poor countries in the status quo. Varying degrees of rivalry and contrasting views of the future have begun to prick the national consciences within the developed states. Further, the smaller and more susceptible rich countries find their interest merging closer to those of the underdeveloped states. This type of realism opens up avenues of salvation for mankind.

UNCSTD, if only for its size, has stirred the attention of the world. It has prompted new insights into the causes of the glaring disparities plaguing mankind. It has stimulated the closer examination of the relationship between science, technology, and societies, and it has bared fundamental questions concerning the future of mankind and the extent to which the global and local environment may be manipulated with impunity. New philosophies are surfacing as a consequence.

A realistic alternative to the arms race has been forwarded as scientists and technologists are being called upon to attack poverty instead of the human race. The concept of science being the common heritage of man is being extended to technology, which signifies the emergence of a new morality within the brotherhood of man. These are fundamental concepts which for expression will require basic changes in the psyche of the human race, and consequently, will be long in reaching maturity or practical fulfillment, but nevertheless they were being voiced by responsible delegates. Probably, UNCSTD is a 'stitch in time' which may save the international community from continuing along the path of global maldevelopment resulting from the misuse of the power of science and technology, and the espousing of bankrupt economic philosophies devoid of strong social probity. ☐

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correspondence

Accounts of East European science are reliable

SIR, — Dr Lorch's attack on Vera Rich (13 September, page 98) is not only graceless: it is also futile. As many an obfuscating party bureaucrat knows to his chagrin, she is a tough and determined person who thrives on reckless abuse and has an uncanny facility for discovering awkward truths behind the glossy facades of official science. In other words, she brings to the readers of *Nature* the best-informed and most reliable account of science in Eastern Europe and the Soviet Union that is publicly available in the Free World. In so doing, Ms Rich complements the correspondingly unsycophantic reports of Colin Norman and David Dickson from Washington, and the sober dispatches that you have recently been publishing from various countries in the Third World. Good work; keep it up.

For it is not by enthusiasm for intergovernmental research projects, nor by the exchange of vacuous academic courtesies between scientists of rival nations, nor by cheerful splurges about marvellous work being done in this or that country, that the pursuit of science will be safeguarded. The fundamental factor for the health of the world scientific community is solidarity in protecting the human rights of scientists of all countries.

I believe that most of my colleagues with recent first-hand knowledge of the state of science in many countries of the Socialist *bloc* would agree that the situation is very largely as Ms Rich describes it, and that this continues to be a matter of the gravest concern for us all. The information that you thus make public in *Nature* is not only essential for those who would combat such evils: it is, by its very publication, of immense influence.

Yours faithfully,

JOHN ZIMAN

University of Bristol, UK

Doctor disappears in Afghanistan

SIR, — I am writing on behalf of the staff of this department, to bring to the attention of our scientific and medical colleagues, difficulties we are having in communicating with a former colleague in the Faculty of Medicine, University of Kabul, Afghanistan.

He is Dr Z. Razban, a medically qualified member of the staff of the Department of Medical Biochemistry in that university. He spent two years with us on a WHO Fellowship to gain experience in teaching biochemistry to medical students and also on research in immunological aspects of protein-calorie malnutrition. When he left us in January 1977, he was well trained in a variety of immunological techniques and had been awarded an MSc based on his research on "Acute phase proteins in children with protein calorie malnutrition" which was also published under this title (Razban *et al. J. Trop. Med. & Hyg.* **78**, 264-266 (1975)).

At that time we understand he was the only doctor in Afghanistan trained in immunology, and on his return he intended to set up an immunological service and to train other doctors and technicians in these techniques, as well as continuing his research and teaching. In order for him to do this successfully it was essential for him to maintain contact with us, and we find it disturbing that for reasons we are unable to discover, this contact has not been maintained. We are certain that Dr Razban would himself have continued to remain in contact with us, and can only assume that he is prevented from doing so by the authorities in Afghanistan.

Yours faithfully,

D.S. JACKSON

University of Manchester, UK

Tumour virus DNA: hazards no longer speculative

SIR, — The critical response (9 Aug, page 444) by Drs Rowe and Martin to the editorial (7 June, page 461) which called on the US Congress to enact recombinant DNA legislation, appears itself to be somewhat misleading.

While it may have been technically incorrect for the editorial to imply that it was unanticipated by tumour virologists that DNA from tumour viruses can cause tumours, it remains true that no one could have known prior to the Rowe-Martin risk assessment experiments (*Science* **203**, 883 and 887; 1979; Israel et al, *Science* in the press) that tumour virus DNA which has been cleaved with restriction enzymes and spliced into plasmids or lambda phage genomes would retain tumorigenicity.

The importance of this finding is the following: up until now opponents of strict recombinant DNA guidelines and legislation have portrayed fears that an aetiological agent could escape the laboratory in a novel biological form as "purely speculative." However, bacterial plasmids and lambda particles do not customarily carry agents of animal carcinogenesis into the ecological niches occupied by their bacterial hosts. It has now been shown by Rowe, Martin and their coworkers that it is a fairly routine matter to construct plasmids and lambda particles with just such a capability. The fact that these splice products are less effective than ordinary polyoma viruses in promoting tumours in the normal hosts of polyoma is irrelevant: the bacterial route for the dissemination and variation of tumorigenic DNA is a qualitatively new one.

The unlikelihood that the Rowe-Martin experiments released any novel tumorigenic vector into the environment is largely due to the fact that these studies were carried out under P4-EK2 containment protocols. However, under the newly revised NIH Guidelines (*Federal Register*, January 15, 1979) such tumour virus splicing experiments can now be carried out under P3-EK1 or P2-EK2 protocols.

It should be noted that the efficacies of both the biological safety cabinets used in P2 and P3 experiments, and of the only approved EK2 host, *E coli* χ 1776, have been recently called into question (*Nature*, 29 March, page 384; S. Levy, technical report to NIH under risk assessment contract; R. Curtiss III, letter to NIH, May 11, 1979).

Since the hazards of releasing into the ecosystem known aetiological agents in new biological forms can no longer be considered speculative, it should now be unacceptable to denigrate efforts by the NIH Recombinant DNA Advisory Committee, and by the public through its unions and government, to tighten containment through guidelines and legislation that extend to industrial as well as academic laboratories.

Yours faithfully,

STUART A. NEWMAN

New York Medical College, New York

Meeting on carcinogen testing is open

SIR, — In the 23 August issue (page 623) Alastair Hay wrote a short article "Press banned from conference" in which he discussed the status of press invitations to a meeting to evaluate short-term in vitro tests for carcinogenicity. The meeting is open to the public as are all such US Government sponsored meetings, and contrary to his headline and article the press has been neither banned, excluded nor specifically invited.

Investigators of the international programme for the evaluation of short-term tests for carcinogenicity will meet in October to present and discuss their results for testing 42 coded chemical samples. Results from a total of 68 assay system investigators will be presented. The meeting will not be comprised of formal presentations but rather of concurrent work groups. During the first part of the meeting five separate work groups will meet to discuss the raw data from similar bioassays. During the second part of the meeting the participants will be grouped into work groups to evaluate the cumulative test data on individual chemicals. Both phases of the meeting will require tedious analysis of large volumes of raw data and review of details of experimental protocols, neither of which is usually of interest either to the scientific community at large or to the press.

The coordinating committee fully recognise the importance of this study as well as the preparation of a final report for general distribution. It was this recognition that led to the decision to use two different mechanisms to bring this final report to the attention of the scientific and regulatory communities and the public.

Firstly a full account of the study will be published including the section on chemicals reviewed and carcinogenicity data on each chemical, results from individual investigators and an overall summary and evaluation. Secondly, a public information meeting has been planned for 3 December at the National Institutes for Health, Bethesda, to present and discuss the complete results of this study. It is the feeling of the coordinating committee that the six weeks between the October and December meetings will be necessary to make a comprehensive and meaningful evaluation.

It is regrettable that there has been such a misunderstanding concerning press participation at the two meetings planned to bring the international trial to completion.

Yours faithfully,

THE COORDINATING COMMITTEE

J ASHBY

B A BRIDGES P BROOKES

I F H PURCHASE T SUGIMURA

● *Alastair Hay writes: My experience, and that of other British science journalists, was that we applied to attend the meeting — only to be told that the presence of journalists might inhibit balanced scientific discussion. I subsequently had a letter from one of the organisers, confirming that no journalist was to be invited. If I misunderstood the committee's real intentions, then I apologise.*

news and views

Arctic animals and climatic changes

from Robert M. May

THERE is a tendency in much of the ecological literature to think of environmental change as taking place either on a very long, or a very short, time scale. Thus attention is given to the way populations, or even entire communities, respond to the major changes in climate that occur on geological time scales. At the other extreme, many studies concentrate on the influence short, year-to-year climatic fluctuations can have on the dynamics of populations (for example, in fish recruitment or locust outbreak). In a recent working paper of the Interim Committee on Marine Mammals of the International Union for the Conservation of Nature (ICMM/1/WP1), Laws, Gordon Clark and Dunbar note that there appear to be significant changes in climate on many intermediate time scales. They emphasise that such systematic environmental changes, particularly in the time range from around a decade to a century, raise important questions for conservation and resource management; these questions have conventionally received little attention.

Most people are familiar with the long sequence of alternations between glacial and interglacial epochs of climate, with a period of about 100,000 years, that has characterised the past million years or so. Mid-latitude temperatures today are 5°-8°C warmer, and sea levels 80 - 100 metres higher, than those prevailing during extreme glacial stages (the most recent being the Würm or Wisconsin glacial maximum, about 18,000 years ago). The World Meterorological Organization and others have used a variety of palaeoclimatic indicators to show that, following recovery from the Wisconsin ice age, the global climate has exhibited systematic fluctuations (albeit within narrower limits than those characterising the ice-age epoch), with expansions and retreats of polar ice and mountain glaciers at intervals of about 2,000-3,000 years. Nested within this is finer-scale structure: stratigraphy and pollen from Swiss and other glaciers shows five cycles of glacial advance and retreat over the past 3,000 years, with temperature minima around 1400-1300 BC, 900-300 BC, AD 400-750, AD 1200-1300 and AD 1600-1850. The 'Little Ice Age' of 1600-1850 is a well-documented historical event, during which temperatures in northern Europe were 1° or 2°C lower than today and stormy conditions prevailed in the North Atlantic. Conversely, some of the intervening climates have been better than today; during the 'Little Optimum' of the 10th, 11th and 12th centuries (with a peak around AD 1080-1180) a Viking colony flourished in Greenland and wine was produced in England.

Laws *et al.* point out that "Within the lifetime of people at present in their 60's there has been a climatic cycle that has affected the economy of a number of countries." This cycle began on an upswing to warmer conditions about 1915, reached a peak in the early 1940s, and returned to colder conditions over the past 35 years or so. This downswing is probably still continuing. Older people's memories of the golden summers of their youth have a measure of objective reality. During this 60-year interval, temperatures in the regions of the West Greenland current, in Baffin Bay, in the waters of Iceland, and around Spitzbergen have fluctuated over many degrees in the atmosphere, and some 2°C in the hydrosphere. In the Antarctic, all stations with long records show trends in mean annual temperature over the past 75 years (1900-1975), of total magnitude 0.3°C at South Georgia, 0.7°C at McMurdo, 1.1°C at Laurie Island in the South Orkneys, and about 1.9°C for the Antarctic Peninsula (British Antarctic Survey Annual Report, 1976-1977). In South Georgia there has been a steady 33% increase in rainfall since 1906, while in the Scotia Sea there have been marked changes in the circulation (BAS Annual Report, 1975-1976). These temperature changes of a degree or two may sound modest, but they represent the transport of enormous quantities of heat.

Many of the grander patterns of human history can be reinterpreted in terms of the systematic climatic fluctuations of the past 3,000 years (see, for example, Ladurie, *Times of Feast, Times of Famine,* Allen and Unwin, 1976). There have undoubtedly also been marked effects on many animal populations, especially in the sea. Unfortunately, little direct evidence is available. One exception is the demonstration by Thoradotir (in *Polar Oceans,* ed. Dunbar, Arctic Institute of North America, 1977) that the primary production north of Iceland decreased greatly by a factor of at least 2 or 3, between about 1965 and 1975, presumably in response to a cooling of the water and the southward extension of the limit of sea ice. Another important study is by Vibe (*Meddr Grønland* **170,** 1; 1967), who uses bone material uncovered by archaeologists in their excavations of Eskimo and Norse settlements in Greenland, and accounts of sales to Greenlanders of rifles and other equipment, to get an idea of the way climatic fluctuations have influenced the population density and geographical distribution of animals in and around Greenland. Vibe considers ringed seals, harp seals, narwhals and white whales, polar bears, walrus, Greenland whale (or bowhead, *Balaena mysticetus*), blue and white arctic fox, ptarmigan, eider, reindeer, musk ox and cod. He broadly distinguishes three periods since the early 19th century. In the "drift-ice stagnation stage" (1810-1960) the climate of northern West Greenland is relatively cold, dry and stable. This climate is favourable for reindeer in central and northern West Greenland, and the population increases; populations of sea mammals and sea birds concentrate at central West Greenland. The drift-ice is also relatively stable throughout the winter off North-East Greenland, where the climate is favourable for the reindeer and the musk ox, whose populations flourish. In the "drift-ice pulsation stage" (1860-1910) the ice of the Arctic Ocean drifts into the Atlantic in larger amounts than before, and the climate becomes warmer with much precipitation and with great fluctuations. The populations of sea mammals and sea birds decrease in central West Greenland, and the wet winters are unfavourable for reindeer and musk ox, whose populations decline. In the "drift-ice melting stage" (1910-1960) the drift-ice of the East Greenland Current decreases, and the climate is relatively warm with some precipitation. Cod multiply in Greenlandic waters, and populations of sea mammals and sea birds increase in northern West Greenland and in East Greenland. The reindeer populations of West Greenland have ample summer grazing, but the winter

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Robert M. May is Class of 1877 Professor of Zoology at Princeton University.

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pastures are often covered by snow and ice, so the population fluctuates. Of particular interest is Vibe's comment that, "Until recently, like all other Arctic animals the Greenland Whale was very numerous during certain periods and less numerous during others. Its distribution and mass occurrence was determined more by movements in the drift-ice than by persecution from Eskimo and European whalers."

These climatic cycles, and the associated changes in the distribution and abundance of animals, have had major effects on the economies of Greenland and Iceland. The gist of Vibe's analysis is that, over the past 60 years, in West Greenland the whole ecosystem moved north with the warming and south again with the cooling, so that a seal-hunting economy was replaced by a cod-fishing economy, the latter now being in some jeopardy. Off Iceland, the herring fishery disappeared northward, and later reappeared in northern coastal waters. Similar events, also involving the Atlantic cod, occurred around Spitzbergen. Discussing long-term trends in the climate, Cushing (*Science and the Fisheries*, Arnold,1977) points to the alternation between the Norwegian herring fishery and that off the Bohuslan coast of Sweden, which has persisted since the Middle Ages, as being associated with the well-documented 100-year periodicity in the wind system of the northern hemisphere. There may be a similar 100-year periodicity in the fishery for the Japanese sardine.

In short, many different lines of evidence and inference suggest that systematic changes in climate take place on different time scales, and with various amplitudes. Laws *et al.* are clearly correct in arguing that "The shorter cycles, terms of a decade and up to half a century cannot be reasonably neglected for purposes of resource management." Two intertwined problems emerge: to achieve skill in forecasting such climatic changes; and to convince resources managers to include these factors in their deliberations. ☐

First discovery of fossil tree shrews

from R.D. Martin

THE recent identification in Indian Sivalik Miocene deposits of fossil material exhibiting clear affinities with modern tree shrews reported by Kaul *et al.* and Vasishat and Chopra in this issue of *Nature* (pages 213 and 214) fills a major gap in our understanding of this unusual group of mammals. The tree shrews achieved prominence some 50 years ago when Le Gros Clark pointed out in a series of papers a number of characters (notably in the morphology of the skull, brain and reproductive system) suggestive of an evolutionary relationship to the order Primates (*Proc.Zool.Soc.,Lond.* **44**, 461: 1924; *ibid* **44**, 1053; 1924; *ibid* **45**, 559; 1925; *ibid* **46**, 1179; 1926). The transfer of the tree shrews (family Tupaiidae) from the order Insectivora to the order Primates was given an international seal of approval by Simpson in his influential classification of the mammals in 1945 (*Bull. Am. Mus. Nat. Hist.* **85**, 1; 1945), and numerous papers followed on the supposed primate-like features of these semi-arboreal, South-East Asian mammals. However, since the 1960s a growing number of investigators have rejected the concept of a specific phylogenetic relationship between tree shrews and primates, and one of the most recent, authoritative primate classifications excludes the tree shrews altogether (Simons *Primate Evolution: An Introduction to Man's Place in Nature,* Macmillan, New York; 1972).

It is probably true to say that the modern consensus is that tree shrews are not related to primates after all. This view is partly based on additional evidence regarding cranial, dental and cerebral morphology along with reproductive characteristics and biochemical aspects. However, reassessment of the methodology used for reconstructing phylogenetic relationships has had a far more fundamental and far-reaching influence on the elucidation of primate evolution. It is, in fact, still true that tree shrews share many striking similarities with primates. But it is also true that all of these similarities can probably be ascribed to one of the following two categories: (1) retention of characters unchanged from the common ancestral placental mammal stock; (2) convergent development of adaptations for life in the trees. A convincing case could only be made for regarding the tree shrews as primates if it were possible to postulate the development of specific characters in a definable ancestral stock derived from the ancestral placentals and giving rise to tree shrews and 'other' primates. Exclusion of the tree shrews from any phylogenetic link with the primates owes as much to increasing attention to the principles of phylogenetic reconstruction as to the discovery of new evidence. As Romer suggested (*Notes and Comments on Vertebrate Paleontology,* University of Chicago Press, 1968), it now seems likely that the tree shrews, rather than providing a living model for the ancestral primate (the view espoused by Le Gros Clark and Simpson), probably provide a better indication of the characteristics of the common ancestor of all living placental mammals.

One major drawback in discussions of the phylogenetic ties of the tree shrews has always been the absence of any clearly identifiable fossil relatives. The Oligocene *Anagale* was, for a while, regarded as an early relative of the tree shrews (Le Gros Clark *The Antecedents of Man,* 2nd ed., Edinburgh University Press, 1971), but has now been assigned to the Lagomorpha (rabbits and hares) (McKenna *Am. Mus. Novit.* **2158**, 1; 1963). Van Valen (*Evolution* **19**, 137; 1965) suggested *Adapisoriculus* as a Late Palaeocene tupaiid, but the fact that this fossil genus has been variously assigned to the marsupials, the insectivores and the tree shrews simply underlines the very generalised morphology of *Adapisoriculus* and the modern Tupaiidae. Strangely, although inferred phylogenetic relationships among mammals have been traditionally based heavily on dental morphology, the dental characters of tree shrews have been barely mentioned in discussions of their evolution. In fact, they exhibit a combination of somewhat unusual specialisations of the anterior teeth with a basically primitive configuration of the cheek teeth. Apart from the loss of one premolar in both upper and lower jaws, they depart only slightly from the pattern postulated for ancestral placental mammals.

The new material from the Sivaliks assigned to *Palaeotupaia sivalicus*, in spite of its fragmentary nature and the absence of any information on the lower jaw, shows clear similarities to modern tree shrews (actually to the Tupaiinae, rather than to the Ptilocercinae). The upper cheek teeth have a similar, basically primitive configuration, while the anterior part of the maxilla together with a premaxilla is similarly drawn out into a rostrum with a small canine posteriorly and two large, well-spaced incisors arranged longitudinally and separated by a large median gap from their counterparts. The upper dentition apparently had the same formula (2.1.3.3) as in all modern tree shrews, and the overall arrangement of the dentition, upper jaw, snout and orbital region is remarkably like that of modern *Tupaia*. Interestingly, although the fragments indicate a skull size comparable to that of the most arboreal modern *Tupaia* species (*T.minor*), the snout is longer and generally more like that of the more typical semiterrestrial *Tupaia* species. This confirms the suggestion (Martin *Man,* **3**, 377; 1968; *Symp. Zool. Soc. Lond.* **33**, 301; 1973) that some of the primate-like features of modern *Tupaia minor* (Le Cros Clark *The Antecedents of Man*), not seen in semi-terrestrial or terrestrial tree shrew species, are independent acquisitions associated with increased arboreal adaptation and as such are of no direct relevance to primate ancestry.

Unfortunately, we still have no fossil evidence relating to the early origins of the tree shrews or their evolution over some 50 million years or more up to the Miocene,

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R.D. Martin is Reader in Physical Anthropology at University College London

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but the new Sivalik finds do at least document the presence of tree shrews of modern aspect in India some 10 million years or so ago, perhaps contemporaneously with such undoubted prosimian primates from the Sivaliks as *Indraloris* (Lewis *Am.J.Sci.* **26** 134; 1933; Tattersall *Postilla* (Yale) **123**, 1; 1968). □

Origin of the Galilean satellites and Jovian rings

from David W. Hughes

REGULAR satellite and ring systems seem to be a common feature of the major planets. These systems are coplanar, the orbits are circular and also the ratio between the orbital radius of a satellite and that of the one immediately interior is a constant, about 1.65 in the case of Jupiter.

The formation of satellites as the planets condensed has often been linked with the formation of the planets as the Sun condensed, and this process has been re-investigated by Prentice and ter Haar in a recent edition of *Nature* (**280**, 301; 1979). These authors return to the hypothesis originally advanced by Laplace in 1796. In the beginning, according to Laplace, the solar and planetary material was in the form of a huge spinning gaseous globe with a diameter larger than that of Neptune's orbit. As this great globe cooled it contracted, spinning faster and faster due to the conservation of angular momentum. A stage was eventually reached when the equatorial centrifugal force became larger than the gravitational force and a ring of matter was left behind, suspended in a circular orbit in the equatorial plane. Due to this mass and angular momentum loss, the proto-Sun could contract a little further, but it soon became unstable again and shed another ring. This process was repeated until the Sun reached its present size.

Now it must be remembered that the present mass of the Solar System is only a very small fraction of the mass of the rings, simply because most of the planets consist of material that is quite rare by normal cosmic standards. Each planet is probably the condensation remnant of a ring of about a thousand times the Earth's mass. Also the spinning gaseous globe was probably strongly turbulent. By considering T. Tauri stars Prentice (*Astr. Astrophys.* **27**, 237; 1973) concluded that the convective velocities in the globe are supersonic and that the turbulent stresses set up by these convective movements are of the order of 30–100 times the normal gas pressure just below the surface. Two things result from this. First, the globe becomes very centrally condensed which obviously leads to a low moment of inertia. Second, a dense shell of non-turbulent gas develops at the surface, this evolving into an equatorial belt as the cloud contracts. It is this belt which is left behind when centrifugal force exceeds gravitational force, and it can be shown that the ring-shedding process repeats itself at a sequence of orbital radii R_n which satisfy

$$R_n/R_{n+1} = (1 + m/M_e f)^2.$$

In this equation m is the mass of the ring, M_e is the mass of the gaseous globe that has just lost the ring and f equals $I_e/M_e R_e^2$ where I_e and R_e are the moment of inertia and radius of the globe.

For the specific case of the Galilean satellites of Jupiter Prentice and ter Haar set M_e to 1.9×10^{30} g the present mass of Jupiter, f to 0.02 and R_n/R_{n+1} to 1.65. This immediately tells us that the mass of each Jovian ring was about 1.1×10^{28} g. If this mass was of solar composition it would have two medium-temperature (~0°C) solid components. The rocky one would have a mass of about 5×10^{25} g and the water ice and rock mixture component would have a mass of about 12×10^{25} g. The globe is warming up as it condenses so each detached ring will have a different temperature. The authors calculate that the temperatures at detachment for Io, Europa, Ganymede and Callisto are 533, 338, 220 and 133 K respectively. It is this temperature difference which causes the chemical variation. Io and Europa with masses of 9×10^{25} and 5×10^{25} g, respectively, have little ice, the high-temperature Io condensation having iron in the form of FeS while for Europa, Fe appears as FeO. The heat released as the ring condenses to form a satellite and as the satellite compacts causes S and H_2O to leak out, leaving sulphur deposits on Io and icy plains on Europa. Ganymede and Callisto condensed at a temperature below the freezing point of water and therefore consist of dirty ice.

By extending this theory, the authors predict that the contracting gaseous globe can shed one or two more rings interior to Io. One has a radius of 2.5 R_p and the other 4 R_p where R_p is the present Jovian polar radius, 6.677×10^9 cm. Amalthea is near the first of these but is spectrally very close to a C-type asteroid and has probably been captured by Jupiter at a later date. These interior gaseous rings probably did not condense to form satellites because there was too little time for the rocks to aggregate into a single body before the gaseous component of the rings dispersed, taking with it the finer dust material. Also the increasing luminosity of proto-Jupiter probably frustrated the settling out process.

Ring shedding cannot proceed indefinitely. The rate of release of gravitational potential energy slows down between 3 R_p and 1.5 R_p in the case of Jupiter. This results in a decrease in the surface temperature and in the supersonic turbulence. R_n/R_{n+1} drops to 1.02, leaving the rings so closely spaced that they essentially merge together to form a continuous disk. The disk does not coalesce into a few single bodies because of its differential rotation. The gaseous component disperses and orbital resonance subsequently changes its structure. It is these resonances which may account for the sharp ring edge seen at 1.9 R_p by the Voyager I and II spacecraft.

No theory in cosmogony is without its rival and the origin of the Jovian rings is no exception. Smoluchowski wrote in the following issue of *Nature* (**280**, 377; 1979) that the rings were probably produced by the tidally induced fragmentation of a non-spherical, mechanically weak carbonaceous body. This disruption is thought to have occurred fairly recently. The solid surface area of the rings has been estimated to be about 20,000 km², this value coming from a comparison between the decrease produced in the charged particle flux by the rings and that caused by Amalthea, both these effects being observed by Pioneer 11. Bombardment by the intense flux of protons in the Jovian Van Allen belts erodes particles. Smoluchowski calculated that ice was eroded at between 50 and 100 Å yr^{-1} and silicate particles at 2–3 Å yr^{-1}. Many small grains are thus eliminated on the time scale of a million years and the Jovian rings are not expected to have a lifetime much longer than this. □

David W. Hughes is a Lecturer in the Department of Physics, University of Sheffield.

Normal fluid density of superfluid ^{3}He-B

from P. V.E. McClintock

NEW high precision measurements of the normal fluid density of liquid ^{3}He-B have yielded some surprises. In particular, it seems that the so-called 'stripped' normal fluid density is independent of pressure, quite contrary to expectation. The experiments were carried out by Archie, Alvesalo, Reppy and Richardson of Cornell University and are reported in a recent issue of *Physical Review Letters* (**43**, 139; 1979).

The hydrodynamic properties of the B-phase of ^{3}He are relatively simple in that the liquid can conveniently be described in terms of a two-fluid model, qualitatively very similar to that which has been used in relation to superfluid ^{4}He for many years. Thus, the liquid is envisaged as being composed of two quite separate but completely interpenetrating fluids: a normal fluid component whose properties are similar to those of conventional liquids; and a superfluid component able to indulge in dissipation-free flow, carrying no entropy, and responsible for much of the seemingly bizarre behaviour exhibited by

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the liquid. Properly speaking, of course, because ^{3}He is a fermion (containing an odd number of fundamental particles) its superfluid phases are more closely analogous to the gas of electrons (also fermions) in a superconducting metal than they are to superfluid ^{4}He, which is a boson (even number of fundamental particles). Thus, the origin of the two-fluid behaviour can be understood in terms of a modified form of the well known BCS theory of superconductivity, with an energy gap separating the ^{3}He atoms which exist in the paired zero-energy state (superfluid component) from the atoms in ordinary unpaired states (normal fluid component). In the A-phase, the energy gap is anisotropic, that is, the effective magnitude of the gap depends on direction within the liquid. It follows that the normal fluid density also depends on direction, rendering the hydrodynamic properties complicated in the extreme. The energy gap and normal fluid density in the B-phase, on the other hand, both turn out to be isotropic, and so the situation is a good deal simpler.

Measurement of the normal fluid density ϱ_n as a function of T/T_c is potentially able to provide a great deal of information about the attractive interaction between the ^{3}He atoms which gives rise to the formation of pairs, and which is therefore responsible for the superfluidity. Here T is the temperature and T_c is that of the superfluid transition. The interaction is in fact extremely complicated, because the very act of forming the pairs in turn modifies the interaction. Much effort is being directed towards gaining an understanding of this so-called strong coupling effect.

The technique used at Cornell for measuring ϱ_n entails shaking the liquid back and forth in a container sufficiently narrow that viscous forces constrain the normal fluid to move, thus contributing to the moment of inertia, whereas the superfluid component slips freely and makes no contribution. The apparatus involves liquid ^{3}He held inside a cell shaped like an inverted mushroom, with a thin coin-shaped cavity in its cap. The stem of the mushroom is a springy beryllium-copper tube which is used not only for filling the sample of ^{3}He but also for providing the restoring force enabling the cap to undergo torsional oscillations. From very careful measurements of the resonant frequency of such oscillations it is possible to deduce the proportion of the liquid which follows the motion of the cap, and hence to calculate the normal fluid density. This type of apparatus has been used before at Cornell. The main importance of the present results is their comprehensiveness, particularly in covering so wide a range of temperatures, achieved by incorporating the experimental cell into a nuclear demagnetisation cryostat.

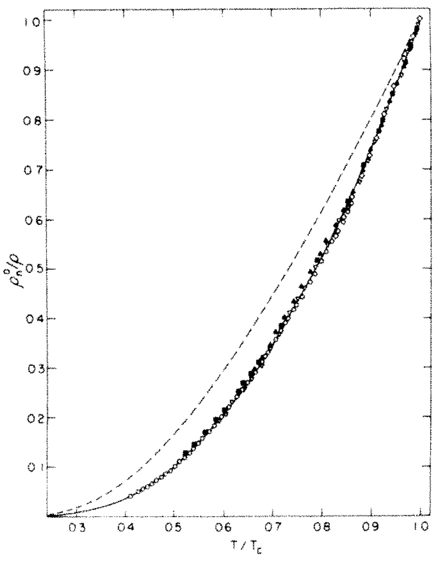

Fig. 1 The stripped normal fluid density ϱ_n^0/ϱ(see text) plotted against T/T_c, where T is the temperature and T_c that of the superfluid transition. On this type of plot the experimental data, recorded for five different pressures between 2 and 29 bar, are clearly pressure-independent and lie well below the weak-field BCS prediction (dashed curve).

Values of ϱ_n/ϱ were measured as functions of T/T_c for several pressures between 2 and 29 bar. Here, ϱ is the total density of the liquid. For any given value of T/T_c, it was found that ϱ_n/ϱ decreased with increasing pressure. The real interest arises, however, when the data are manipulated to give what the authors describe as the "stripped normal fluid fraction", ϱ_n^0/ϱ: in effect, this amounts to removing the known effect of the Fermi-liquid molecular field, the interaction which is present in the normal (non superfluid) liquid at temperatures above T_c. Any deviations between ϱ_n^0/ϱ and predictions based on the BCS approach can then be attributed to strong coupling effects. The result of this operation is shown in Fig. 1. It is immediately apparent that, within the (remarkably small) random experimental error, ϱ_n^0/ϱ is completely independent of pressure. Also, in that there is a sizable difference between the data and the BCS theoretical curve, it appears that there are substantial strong coupling effects, as expected. It would seem, therefore, that the strong coupling effects are themselves independent of pressure.

This is a most unexpected conclusion. It seems to be at variance with other measurements of the strong coupling corrections based on specific heat or sound attenuation data, but to be in reasonable agreement with measurements based on other types of hydrodynamic experiment, once these have been readjusted in the light of the latest temperature scale. Devising an explanation of these results will be an interesting challenge for theorists working in the area and should eventually lead to an enhanced understanding of both superfluid phases of ^{3}He. ☐

P.V.E. McClintock is a Senior Lecturer in the Department of Physics at the University of Lancaster.

All sorts of protoplasts

from Edward C. Cocking

ONE of the most refreshing aspects of protoplast research is that there always seems more to do. At the recent 5th International Symposium*, which covered most aspects of plant, fungal and bacterial protoplasts, it was clear that many laboratories are now using this novel cell system for many basic and applied investigations. Some of the basic studies discussed included pioneering work by L. Vigh and his colleagues at the Biological Research Centre, Szeged on the incorporation of spin-labelled fatty acids into the plasma membranes of intact wheat protoplasts. Significant differences were found between the microviscosity of membranes of cold-resistant and cold-sensitive wheat cultivars indicating that membrane fluidity may be directly involved in avoiding freezing injury — perhaps growth substance workers could find this a useful probe for early effects on the growth of single cells.

With the current interest in genetic manipulation, it was understandable that there should be a major emphasis on the fusion of protoplasts for somatic hybridisation, and on the use of protoplasts for transformation. A particularly useful feature of this meeting was that it afforded the opportunity of comparing progress between prokaryotic and eukaryotic systems. Classical genetic information transfer in bacteria is unidirectional, contrasting with the products of induced protoplast fusion which produces a new entity containing the whole genome and cytoplasm of two or more bacterial cells. L. Alföldi and his colleagues (Biological Research Centre, Szeged) highlighted the limitations of classical methods of genetic analysis when applied to such bacterial protoplast systems. The industrial potential of these interesting recombinants has not, apart from work on *Streptomyces*, been fully exploited yet.

However, the outlook for fungal protoplasts is more encouraging. Many workers have investigated the fusion of the protoplasts from a wide range of fungi. New genetic variability is being produced as a consequence of fungal protoplast fusion, particularly between various *Candida* species that are important in single cell protein production. These basic studies are providing a foundation for many other industrial applications (see Peberdy *Proc. 3rd Int. Symp. Gen. of Industrial Microorganisms*, 1978). Better strains of *Trichoderma* for improved cellulase production are being developed and brewing yeasts could benefit from the readiness with which polyploid strains can

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*This meeting on Microbial and Plant Protoplasts was held in Szeged, 9-14 July, 1979, jointly organised by the Department of Microbiology, Attila Jozsef University, and the Institutes of Genetics and Plant Physiology, Biological Research Centre. The proceedings will be published by Pergamon.

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be produced by protoplast fusion methodology. These studies and a refinement resulting in interspecific mitochondrial transfer (L. Ferenczy, Atila Jozsef University, Szeged; R. Dales & J.H. Croft, University of Birmingham, Birmingham), and the now well-documented transformation of yeast protoplasts by chimaeric plasmids, have fully established fungal protoplasts in the forefront of genetic manipulation.

Studies on the reversion of fungal protoplasts were however, not neglected (O. Necas, J.E. Purkyne University, Brno) and the report on chitosomes and the origin of chitin microfibrils (Bartnicki-Garcia, University of California, Riverside) showed the usefulness of protoplasts for elucidating the details, both structural and biochemical, of cell wall synthesis at a naked plasma membrane surface.

Close parallels exist with plant protoplasts in this respect, but the more exacting developmental processes involved in whole plant regeneration have restricted the range of species for plant genetic manipulations. Several workers emphasised the difficulty in releasing the probable inherent totipotency of single cell cereal cultures—isolated cereal protoplasts are the 'acid test' for such regeneration capability. Chinese workers reported limited success in obtaining a few cell divisions in cultures of wheat leaf mesophyll protoplasts. Protoplasts isolated from suspension cultures derived from callus of pearl millet immature embryos were reported to divide to form embryoids and subsequently whole plants (I.K. Vasil & V. Vasil, University of Florida, Gainesville). Several participants doubted whether this system would be reproducible, and also whether the approach adopted would be applicable to the main cereal crops; but this report will no doubt greatly stimulate work on cereal protoplasts.

Virus replication studies using plant protoplasts do not depend on such a full developmental sequence; and have provided an important clue to the various resistance barriers that may be by-passed using protoplasts: I. Takebe (Nagoya University, Nagoya) reported that radish mesophyll protoplasts are readily infected by brome mosaic virus which shows no evidence of infection in radish leaves.

A somewhat similar breakdown of infectivity barriers is suggested by the reports of the transformation of plant protoplasts by isolated *Agrobacterium* plasmids — in future it may prove possible to transform protoplasts of monocotyledons although *Agrobacterium* itself infects dictotyledons only. If this is coupled with the ability to regenerate whole plants, a sound basis could be provided for the transformation of cereals.

Understandably most of the somatic hybridisation work using plant protoplasts has utilised more culturally amenable species. Although several somatic hybrid plants have been obtained between species that have not, as yet, been crossed sexually this has not resulted in the production of useful amphidiploids. It is still not clear whether chromosome elimination in heterokaryons of disparate species will result in cybrid formation, with the possibility of mitchondrial recombination as suggested from the restriction nuclease analysis of G. Balliard and G. Pelletier (University of Paris-Sud, Orsay) or regeneration of plants with only very limited gene transfer. There is obviously the possibility of such somatic transformation using disparate plant protoplast combinations (D. Dudits *et al.*, Biological Research Centre, Szeged).

There was general agreement that only continuing research on all these various aspects of protoplasts will provide an adequate foundation for applied developments. □

Edward C. Cocking is Professor of Botany at the University of Nottingham.

Twenty years of pheromomones

by Mary Lindley

THE exchange of chemical signals between living organisms has proved to be far more complicated than it seemed twenty years ago, when pheromones were first defined as substances responsible for chemical communication among individuals of the same species. Through the combined efforts of chemists, ecologists and physiologists, concentrating chiefly on insects, the original idea that one substance served one species has given way to the realisation that the pheromome secreted by a particular species is a characteristic mixture of components. Each component has a particular function in communication within and between species, affecting the behaviour involved in, for example, attraction, inhibition and alarm. Electrophysiological studies of insect chemoreceptors, such as those on antennae, have shown that different cells respond to different components, and that the central nervous system interprets the resulting signal according to the level of excitation of the various cells.

The realisation that there is more to chemical communication than sexual attraction has necessitated a more extensive classification of the chemicals involved. When individuals of different species communicate chemically, the signal is a kairomone if the receiver benefits, as in the case of certain predators of bark beetles which aggregate in response to secretions from their prey. If the sender benefits, however, the signal is an allomone, such as those produced by orchids of the genus *Ophrys* to attract the bees that pollinate them. Some of the contributors to the growing understanding of chemical communication gathered recently in Sweden*. Among the inevitable and essential accounts of further pheromone components isolated and synthesised, there were some descriptions of newly recognised chemical relationships and progress reports on the application of pheromones in pest control.

As sex attractants, pheromones have been considered primarily to be produced by females to attract males, but this is not always the case, and D. Schneider (Max-Planck-Institut für Verhaltenphysiologie, Seewiesen) described one produced by male monarch butterflies (family Danaidae). Through thin hairy organs, known as hairpencils, they release a pheromone to prepare the females for mating. Its principal component is a heterocyclic ketone — danaidone — synthesised from precursor alkaloids taken up by the butterflies from certain food plants. Synthesis of danaidone also often requires contact between the hairpencils and the male's wing gland. Together with cardiac glycosides, the alkaloids also serve as chemical defence agents which render the butterflies unpalatable to some of their predators.

R. Baker (University of Southampton) believes that some of the most successful West African predatory ants have become specialised so that their pheromones, used in alarm and recruitment of nest mates, are not detected by their prey (*Experientia* in the press). The workers of some species of myrmicine ants can move freely within the galleries of termites before attacking the inmates and carrying them to their own nests. But when the Southampton group exposed termites to workers of related species of ants, the termites retreated. Bioassays and analysis of the secretion of the mandibular gland in various myrmicine ants have shown that the specialised or semi-specialised predators produce aliphatic alcohols, which do not affect termites, instead of carbonyl or other compounds, which are usually repellant to termites. Baker suggested that the secretions of the ant's mandibular glands may provide clues by which termites recognise predators, and that the most successful predators are those producing secretions the termites cannot detect.

When attention turned to pest control, there were enthusiastic reports of some of the programmes that have been set up throughout the world. The general response, however, was less enthusiastic, with discouraging references to unobtainable patents and the strict standards of environmental agencies more

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*A conference entitled "Chemistry of Insects: Chemical Interaction and Communication" was held in Borgholm, Sweden on 13-17 August 1979. It was organised by G. Aulin-Erdtman of the Swedish National Committee for Chemistry of the Royal Swedish Academy of Sciences, and sponsored by Euchem and the Swedish National Committee for Chemistry.

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familiar with toxic pesticides than behaviour-modifying chemicals. Although nobody could foresee pheromones alone taking care of pest control, there was nevertheless a general hope that they would decrease the present dependence on pesticides.

Mass trapping — large scale capture of pests in traps baited with pheromones — forms the basis of several programmes. A. Shani (Ben-Gurion University of the Negev, Be'er Sheva) pointed out that Israeli farmers spray their cotton fields with pesticides less often than was necessary before mass trapping of the Egyptian cotton leaf worm, *Spodoptera littoralis*, began in 1975. Mass trapping is also the basis of the Norwegian campaign against the spruce bark beetle, *Ips typographus*. Although the beetle is usually a valuable member of the forest ecosystem, accelerating the decay of dead wood, its population has grown so rapidly during the past 10 years that much of Norway's spruce forest is threatened. L.A. Riege (Borregaard Industries Ltd, Sarpsborg) felt that there were signs of success in the catches of the 600,000 traps set up this year with a lure of bark beetle pheromone.

G.N. Lanier (State University of New York, Syracuse) reported progress in trials with the aggregation pheromone of the European elm bark beetle, *Scotylus multistriatus*, the carrier of the fungus that causes Dutch elm disease. Lanier has seen a decline in the incidence of diseased trees in trial areas during the past year. He spoke enthusiastically of the potential of this sort of control programme if accompanied by other precautions, such as the proper removal of infected wood.

L. Rechav (Rhodes University, Grahamstown) has been attacking the tick *Amblyomma hebraeum* by painting cattle with a mixture of tick pheromone and acaricide. Small areas of the mixture attract unfed nymphs and adults, killing them before they bite the cattle, thus reducing the chance that they will spread disease. However, this method has the disadvantage that tick assembly pheromones are species specific and remain effective on the cattle for only three days, precluding widespread application at present.

The aim is not always to trap and kill insects, but sometimes to confuse them by filling the atmosphere with pheromones so that the males cannot find the females. The only effective way to monitor the results of such methods is to count the larvae that hatch in a test area, and the results can be distorted by the arrival of gravid females from untreated areas. In tests of the efficacy of mating disruption by pheromone inhibitors of the striped rice stem borer, *Chilo suppressalis*, at the International Rice Research Institute in the Phillipines, this problem has been overcome by the use of large field cages. P.S. Beevor (Tropical Products Institute, London) said that it had been possible to quantify rates of release that would disrupt mating up to 100% in this way. The next stage will be to microencapsulate the inhibitors for slow continuous release and set up trials in the open field. As with similar test programmes, however, the outcome cannot be predicted.

A third practical application of pheromones is to attract pests for population monitoring. Baker reported the early stages of a programme to monitor the pine beauty moth, *Panolis flammea*, a pest of lodgepole pine in Scotland. Once the most effective formulation of pheromones has been worked out, traps will be set up to capture sufficient moths to reflect variations in the size of the populations.

Participants were glad to note that monitoring is already routine in many areas, with commercially produced traps, pheromones and dispensers being used to forecast the size of pest populations. On the basis of the numbers caught in the traps, farmers and their advisors decide how often to spray with pesticides, and there has been a general reduction in the frequency of spraying. Pests being monitored in this way include the pea moth, *Laspeyresia nigricana* in England and northern Europe, the codling moth, *L. pomonella* throughout Europe, *S. littoralis* is Israel and the pink bollworm, *Pectinophora gossypiella* in California.

Pheromones may have uses beyond the continuing battle against pests. The declining population of honey bees, *Apis mellifera*, in Britain and the unattractive nature of arable crops have stimulated attempts to use pheromones to bring the two together for pollination of the crops. J.A. Pickett (Rothamsted Experimental Station, Harpenden) described how this work has brought an unexpected insight into the way the bee apparently controls its output of pheromone components. The Nasunov gland, between the sixth and seventh segments of the body, produces several components, of which (E)-citral is the most active in attracting other bees, although it is very volatile and present in very small quantities. Pickett and his colleagues have found that the gland has a highly specific enzyme system that converts geraniol to (E)-citral. He suggested that this system controls the release of (E)-citral, ensuring that it is present in sufficient quantity to be active.

The way in which insects synthesise their pheromone components and release them in precise proportions are problems for the next twenty years, together with the mode of action at the receptors and the subsequent fate of the pheromone. Meanwhile there are plenty more pheromones to be isolated from an abundance of insects. □

Mary Lindley is an Assistant Editor of Nature.

A hundred years ago

The French Association for the Progress of Science has taken a bold step and decided that its session for 1881 will take place in Algiers. To avoid the numerous inconveniences of the strong heat which prevails all over Algeria in the month of August, it has been decided that the meeting should be held in April, during the Easter recess.

This happy result of the deliberations must be attributed to the personal exertions of M. Albert Grévy, the brother of the President of the French Republic, who holds the post of Civil Governor-General of Algeria.

It is supposed that the new scientific establishment whose formation has been decreed this year, will be formally inaugurated on this occasion, and a scientific movement of some importance will take place in the colony.

The *Akhbar* announced a few days back that a geographical society is being organised in Algiers.

In the meantime a number of representatives headed by Algerian senators and deputies will make a tour of exploration during the month of October. They will start at the end of September, as we announced some weeks ago. They will witness an agricultural and horticultural exhibition, which is to be held at Bone, for the whole of Algeria and Tunisia, and which will be held in Algiers in 1881.

The most successful lecture this year at Montpellier, was organised by the Languedocian Society of Geography. MM Solcillet, Brau de Saint Pol Lias, Director of the Sumatra Exploring Company, and other explorers or intending explorers, appeared before the public on that occasion. M. Rabaut, the President of the Society of Geography of Marseilles, and the commercial agent for the Sultan of Zanzibar, gave most interesting details of the several explorations at present going on in that part of the Dark Continent.

The lecture on the progress of electricity was given by M. Denayrouze, of Jablochkoff candle notoriety. The speaker tried to show that Jamin's candle ought to be superior to the light which is spreading so largely in Paris and in London.

The British Association

Report of the Committee for exploring Caves in Borneo. Drawn up by Dr. J. Evans F.R.S. — The Committee report that with the grant of 50 l. from the Association, a similar grant from the Royal Society, and a farther sum of 200 l. from private sources, they have been able to prosecute an examination of various caves in Borneo, under the superintendence of Mr. A.S. Everett, who has devoted himself to the task for a period of nearly nine months.

The final report upon his work has not yet been received, but it appears from his letters and from the specimens which have been transmitted to this country, that nothing of special interest either from an anthropological or a geological point of view has resulted from his explorations.

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articles

A binary star model for SS433

D. S. De Young
National Radio Astronomy Observatory, Charlottesville, Virginia 22901

Geoffrey Burbidge
Kitt Peak National Observatory, Tucson, Arizona 85726

The novel features recently observed for the strong emission line object SS433 can be explained by a model in which SS433 is a binary of a massive early-type star and a magnetic white dwarf.

RECENT observations by Margon *et al.*[1,2] and Liebert *et al.*[3] of the strong emission line object SS433 have revealed some extremely interesting and unusual features. Most prominent among them is the presence of strong emission lines of hydrogen and neutral helium which show simultaneous, large redshifts and blueshifts of up to $+50{,}000\ \mathrm{km\ s^{-1}}$ and $-30{,}000\ \mathrm{km\ s^{-1}}$. These shifts are relative to a nearly stationary line system[1] of velocity $\leqslant 180\ \mathrm{km\ s^{-1}}$. The detailed line structures are complex and show smaller velocity variations on a scale of days[1,3], as well as variations of linewidth from 50 to ~200 Å. A second unusual characteristic is that the red- and blueshifted systems merge to a common velocity[4] of $\approx +10{,}000\ \mathrm{km\ s^{-1}}$ with an apparent period of 160 ± 3 days[2,4], thence returning to high red- and blueshifted values. Moreover, there seems to be no evidence of eclipsing of the high velocity features during a period; the period is consistent with a sinusoidal form, and there may be evidence for non-Keplerian motion in the high velocity features[4]. Margon *et al.*[1] deduce a distance to SS433 of $\geqslant 3.5$ kpc, giving $M_v \approx -3.5$. To a limit equivalent to that of the Sky Survey plates, the object appears stellar and is considerably reddened[1]. A search for optical polarisation[3] has produced an upper limit of 0.1%. Seaquist *et al.*[5] have observed SS433 at radio frequencies over a period of 18 months and found variations on a scale of hours to weeks at centimetric wavelengths, with changes of flux of up to factors of ~2.5 (200 to 500 mJy) on the longer time scale. Neither linear nor circular polarisation was detected at 2.7 and 8.1 GHz, and spectral information available suggests a non-thermal origin for the radio emission. VLBI observations of SS433 at 21 cm show no structure on a scale of several milliarc seconds down to a limit of ≈20 mJy (R. Sramek, personal communication). Finally, the object may be associated with the X-ray source A1909+04[1].

Constraints on models

A viable model for SS433 must primarily account for: (1) the large luminosity in the emission lines of $\sim 10^{34}\ \mathrm{erg\ s^{-1}}$; (2) the large redshifts and blueshifts relative to a nearly stationary system; (3) the periodic merging of these features to a value of $+10^4\ \mathrm{km\ s^{-1}}$; (4) the stellar appearance of the object; and (5) the observed emission at radio and X-ray frequencies. For a brightness temperature of $\sim 10^4$ K; (1) implies that the Hα emitting region must have a size $\sim 10^{12}$ cm if the emission comes from an optically thick gas, as appears to be the case[3]. This eliminates accretion onto a neutron star, since the accretion radius is much smaller than 10^{12} cm. Similarly, Zeeman splitting in the magnetosphere of such a star to produce (2) is eliminated because no significant optical polarisation is present[3]. Terlevich and Pringle[6] have proposed a model for SS433 which involves accretion onto a $3 \times 10^5 M_\odot$ black hole, while Fabian and Rees[7] have suggested that oppositely directed high-speed jets emanate from a central source of unknown nature. Both of these models, in their present form, can account for the observed characteristics except for the periodic merging to a high velocity required by constraint (3).

A binary star model

We propose that SS433 is a member of a class of astrophysical systems whose existence has been confirmed by observations; that is, we suggest that SS433 is a binary star system comprised of a massive early-type star and magnetic white dwarf. As we describe below, the observed characteristics of the system can arise if the rotating white dwarf possesses gas imbedded in a dipole field either aligned with or at large obliquity to its spin axis, and with the plane of the orbit lying nearly in the plane of the sky. Precession, but more probably nutation, induced by dissipative interaction with the primary can give rise to the apparent periodicity.

Production of the shifted emission lines: The general characteristics of magnetic white dwarf stars have recently been reviewed by Angel[8]. Typical masses and radii are $\sim 1 M_\odot$ and 10^9 cm. Inferred surface magnetic fields range from 10^7 G to $>10^8$ G; the upper limit of the surface field is uncertain, and 10^9 G is not excluded. A firm upper limit is reached at 10^{12}–10^{13} G when the magnetic field energy density reaches balance with the hydrostatic pressure. Rotation periods vary from ~1 min to $\leqslant 100$ yr, and a considerable offset between the rotation axis and the magnetic field symmetry axis is often indicated. Simple dipole field structure is evident.

For SS433, we must first obtain an estimate of the energy density in the emission line regions. This is not well determined if the region is truly optically thick because of ambiguity in the number density n. If, however, one assumes that all of the emission eventually escapes in the wings of the lines, a value for n may be obtained [we are indebted to R. L. Brown for pointing this out]. Terlevich and Pringle, apparently following this reasoning, arrived at $n \sim 10^{12}\ \mathrm{cm^{-3}}$, although this number is by no means secure except as an upper limit. The presence of large optical depth and the absence of pressure broadening of the lines implies n to be $\sim 10^{10}$–$10^{12}\ \mathrm{cm^{-3}}$, in which case $nkT \sim 10^{-2}$–1 in the emission line region. These energy densities may then be compared to the magnetic field strengths at a distance of $r = 10^3 R_* = 10^{12}$ cm, where R_* is the radius of the magnetic white dwarf. A dipolar surface field of 10^9 G will have a strength of ~1 G at this radius, and thus the line emission regions at 10^{12} cm can be dynamically controlled by the magnetic field. Gaseous

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material has the easiest access onto magnetic field lines in the polar cap regions, and thus we suggest that the emission lines can arise from material forced into near co-rotation at $r \sim 10^{12}$ cm above the two polar caps of a dipole field offset at nearly 90° from the rotation axis.

The velocities required are $\sim 0.15c$, which at $r = 10^{12}$ cm, implies a rotation period of ~1,500 s, which is well within the observed range for such stars. In order for the star not to be disrupted at this angular velocity, we require $\omega^2 R_* < GM/R_*^2$, or $R_* < 2.5 \times 10^{10} M_\odot^{1/3}$ cm, a condition easily satisfied by a white dwarf. Neglecting for the moment the orbital considerations, material in the polar cap regions will be seen to move towards, or away from the observer with a frequency shift given by

$$\nu_{\text{em}}/\nu_{\text{obs}} = \gamma(1 - \beta \cos \theta) \qquad (1)$$

where θ is the angle between the velocity vector and the line of sight.

In addition to the co-rotation, the emission line regions also undergo centrifugal acceleration. At $r = 10^{12}$ cm material in the polar cap regions must be accelerated away from, rather than accreted onto the star, since $\omega^2 r = GM/r^2$ at $r = 2.45 \times 10^{10}$ cm. Thus material injected onto field lines at radii less than $10^3 R_*$ will acquire a radical velocity

$$v_r(R) = \int a(r)\,\mathrm{d}t = \int \frac{a(r)}{v_r(r)}\,\mathrm{d}r;$$

$$a(r) = \omega^2 r - GM/r^2 \qquad (2)$$

If injection occurs near the equilibrium radius, then equation (2) yields $v_r \sim \omega r \approx 0.1c$ at $10^3 R_*$, comparable to the co-rotation velocity. Because of this centrifugal acceleration, gas at 10^{12} cm may not be in exact co-rotation if $\rho v_r^2 > B^2/8\pi$. The magnitude of this effect is unknown due to the uncertain values of the gas density and the magnetic field strength at the surface of the white dwarf. The absence of forbidden lines[9] implies a lower limit to the gas density of about 10^8 cm^{-3}; a surface field of $\sim 10^{11}$ G will provide exact co-rotation in this case. In either case, velocity shifts of +50,000 km s^{-1} and −30,000 km s^{-1} are achieved from equation (1) with a value of $\gamma = 1.033$ and a maximum value of $\cos \theta$ of ±0.5. Thus the observed velocity extremes result when the plane containing the polar caps is inclined at about 30° to the plane of the sky.

The total amount of gas in the emitting region is small; $m_g \sim 10^{-12}$–$10^{-10} M_\odot$, depending on the uncertain value of n, with $10^{-10} M_\odot$ being an upper limit. A similar value for the mass was obtained by Fabian and Rees. Beyond the line emitting region the expanding gas cools in the rapidly diverging field, and the observed line widths result from absorption by the gas of much larger column density which lies above the polar caps; that is, emission is seen only when the poles lie near the sky plane; at other times it is blocked by the larger volume of cooler gas in the line of sight above the polar region facing the observer. The small mass of gas in the line-emitting region can be produced by activity near the surface of the white dwarf triggered by infall of material at lower radii[8]. The infall may arise from direct accretion, or more probably from gas trapped on closed field lines at earlier epochs. In either case ejection is possible only along the open field lines at the poles, and the mass injection rate is $M \approx R_{\text{em}}{}^2 \rho_{\text{em}} v_{r,\text{em}} = 10^{-14} M_\odot\,\text{s}^{-1}$, where the subscript 'em' refers to conditions in the emission line region. This small value is at the low end of estimates for accretion onto magnetic white dwarfs[10].

Origin of the periodicity of the emission lines: With the value of $\gamma = 1.033$ needed to obtain the maximum and minimum velocities of the emission lines, the observed periodic nature of these lines follows directly if the plane containing the magnetic poles moves through an angle of ~30° with a period of ≈160 days. This shifts the plane of rotation into the sky plane with a uniform merging of the emission line velocities to a common value of $+10^4$ km s^{-1} given by the transverse Doppler effect (equation (1), $\theta = 90°$) at $\gamma = 1.033$. For a near 90° offset of magnetic and spin axes, this implies a 15° precession or nutation angle of the spin vector. During that portion of the period when the pole caps lie in the sky plane, emission is seen from both polar regions. One result of this configuration is that the emission lines will vary in intensity with a period of $\sim 10^3$ s when the lines are at the extremes of their velocity shifts, but that no pulsation will be observed when the lines merge to a common velocity.

Another possibility is that the magnetic field axis is aligned with the spin axis, and that gas is accelerated outwards along the open field lines above the poles. In this case no variation in intensity would be observed, since the line emitting regions would be at all times visible to the observer. Preliminary results from MMT observations[11] indicate little variation on short time scales (~1 to 100 s) and may favour an aligned spin and magnetic axis model. However, such a model also requires a 15° precession or nutation of the spin vector.

The orbital characteristics of the white dwarf can only be determined indirectly, since its magnitude at 3.5 kpc is $m_v \approx 26$–29. A commonly observed configuration is for the spin and orbital angular momenta to be near alignment; this geometry would place the orbital plane of the white dwarf roughly in the sky plane and account for the observed lack of eclipsing of the emission lines. It can easily be shown that precession or nutation driven by the usual gravitational torques upon a non-spherical magnetic dwarf cannot produce the required period, even for extreme differences in the principal moments of inertia[12]. To do this would require either an immensely massive primary ($10^6 M_\odot$) or an orbital radius much smaller than the dimension of the line emitting regions. However, either viscous, dissipative coupling or magnetic coupling between the white dwarf and the primary will also exert a torque upon the white dwarf. The maximum co-rotation radius of the magnetic field surrounding the white dwarf is $\sim 10^{13}$ cm, thus an orbital radius of ~10 times the size of emission-line regions will provide strong coupling between the stars.

To see if such coupling can produce the required period, we use the standard Euler equations[13] where the Euler angles ϕ, θ and ψ have their usual meaning. We assume that the star is not grossly deformed, so that the moments of inertia $I_1 \approx I_2 \approx I_3 = I$. If T is the total torque exerted by the coupling upon the star, $\vec{T} \approx \vec{F}_{\text{d}} \times \vec{R}$ where F_{d} is the drag force and R is the radius of the orbit. A condition of constant precession, namely $\ddot{\theta} = \dot{\theta} = 0$, and negligible spin down, $\ddot{\psi} \approx 0$, can be found with the required precessional frequency $\dot{\phi} \approx T \sin \theta/(\omega_s I) \sim 10^{-7}$ s^{-1}, where $\omega_s = \dot{\psi} \sim 10^{-3}$ is the spin of the star and $\theta \sim 15°$. However, in this case the third Euler equation yields $\ddot{\phi} \sim 10^{-3} \dot{\phi}$ which implies a very rapidly evolving set of orbital parameters.

A more stable system seems much more probable, and hence we consider nutation ($\dot{\theta} \neq 0$) and slow variation of the other orbital characteristics. For small obliquity, negligible spindown, and a precession period long compared to nutation period, a periodic solution $\ddot{\theta} \approx -T\theta/I$ exists, with nutation frequency $= (T/I)^{1/2}$. Setting this equal to the required value of 10^{-7} s^{-1} yields $F_{\text{d}} = 10^{24}$ for $R = 10^{13}$ and a white dwarf mass and radius of 1 $M_\odot$ and 10^9 cm, respectively. If the coupling is due to viscous drag, the shear between the co-rotating gas and the atmosphere of the primary will be proportional to the gas density, the square of the relative velocity, and the surface area of the shearing region: $F_{\text{d}} = \varepsilon \rho v_{\text{rel}}{}^2 A$, where $\varepsilon \leqslant 1$ is a measure of the coupling efficiency. For $V_{\text{rel}} \sim 10^{10}$ cm s^{-1}, $A \sim 10^{24}$ cm^2, $\varepsilon n_{\text{gas}} \approx 10^4$. Thus for gas densities in the outer envelope of the primary as low as 10^6 cm^{-3}, a 1% coupling efficiency will provide the necessary drag force. This efficiency is higher than that normally encountered in purely gas dynamical situations, and its enhancement may be attributed to magnetic coupling at $R = 10^{13}$ cm.

Another contribution to coupling between the stars arises from interaction between the magnetic fields of the primary and secondary. The luminosity and emission line spectra observed at +180 km s^{-1} imply that the primary is a fairly early type star with mass $\sim 1 - 10 M_\odot$. If, for example, the primary were an Ap star with surface field ~30,000 G and radius 5×10^{11} cm, the magnetic torque induced upon the white dwarf is $T \sim (4\pi/3)B_2 r_2^3 B_1 (r_1/R)^3 \sim 1 \times 10^{37}$, where the subscripts 1 and 2 refer to the primary and to the white dwarf, respectively. The resulting drag force is $F_d = T/R \sim 10^{24}$ dyn, which is the required value. If such coupling is dominant it would imply that SS433 is similar to the model of AM Her suggested by Stockman *et al.*[14]. Radio emission can easily arise in the lower regions of the white dwarf magnetosphere, with the X-ray emission being produced by infall onto the stellar surface[8].

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Oxidation of CS_2 and COS: sources for atmospheric SO_2

Jennifer A. Logan, Michael B. McElroy, Steven C. Wofsy & Michael J. Prather

Center for Earth and Planetary Physics, Harvard University, Cambridge, Massachusetts 02138

Oxidation of CS_2 and COS initiated by reaction with OH can provide a source of atmospheric SO_2 as large as 12 Mtonnes S yr^{-1} and may represent the dominant source of SO_2 in remote regions of the marine troposphere.

EMISSION of sulphur associated with combustion of fossil fuel represents a major source of atmospheric pollution[1,2], contributing ~65 Mtonnes S yr^{-1}. Although the chemistry of sulphur in polluted atmospheres has been studied extensively, our knowledge of the natural cycle remains rudimentary. Measurements[3,4] at remote marine locations in the Southern Hemisphere indicate that the atmosphere contains a relatively constant background level of COS and SO_2. We shall argue here that SO_2 is derived at least in part from oxidation of COS, with additional sources due to CS_2 and dimethyl sulphide (DMS).

Sulphurous compounds in the atmosphere

It seems probable that the chemistry of sulphurous compounds in the troposphere should be dominated by reactions involving OH [5–7]. The hydroxyl radical is formed by reaction of $O(^1D)$ with H_2O, and is removed primarily by reaction with CO and CH_4. Results of model calculations for the concentration of OH are given in Fig. 1. We believe that concentrations shown here should be reliable to within about a factor of 2, and uncertainties of this magnitude should not qualitatively affect the arguments which follow.

Sulphur dioxide may be removed from the atmosphere by various heterogeneous processes[8], and by gas phase reactions[5,9] involving OH, HO_2 and CH_3O_2:

$$SO_2 + OH + M \rightarrow HSO_3 + M \qquad (1)$$

$$SO_2 + HO_2 \rightarrow SO_3 + OH \qquad (2)$$

and

$$SO_2 + CH_3O_2 \rightarrow SO_3 + CH_3O \qquad (3)$$

The products in reactions (2) and (3) have not been measured directly, and these reactions may possibly involve formation of adducts. Reaction (1) has been studied by several groups[5,10], using a variety of techniques, and its rate constant is probably known to within about a factor of 2. It constitutes a major, perhaps dominant, path for removal of SO_2. Reaction (3) could be comparable to reaction (1). Considerable uncertainty attaches, however, not only to the mechanism of reaction (3), but also to the concentration of atmospheric CH_3O_2. Reaction (2) is of minor importance.

Studies of SO_2 in polluted air parcels[11,12] suggest an atmospheric lifetime of about a day. Calculations presented here for the marine atmosphere, using the kinetic data summarised in Table 1 but omitting heterogeneous reactions, indicate a lifetime of about 8 days. The difference may reflect in part the role of heterogeneous chemistry, in part omission of processes in polluted air which might be expected to result in higher concentrations of radicals (RO, RO_2). The calculations presented here were carried out using a one-dimensional photochemical model, as described by Logan *et al.*[13], with modifications to their chemical scheme as given in Table 1. Vertical profiles for O_3, CO, CH_4 and H_2O were specified using available observational data and calculations were performed for latitudes 45°N, 15°N, 15°S and 45°S.

Significant quantities of SO_2 may be formed during oxidation of COS and CS_2. It appears[6] that oxidation of COS should be initiated by reaction with OH,

$$OH + COS \rightarrow SH + CO_2 \qquad (4)$$

Subsequent steps may involve production and removal of SO:

$$SH + O_3 \rightarrow HSO + O_2 \qquad (5)$$

$$SH + O_2 \rightarrow SO + OH \qquad (6)$$

$$SO + O_2 \rightarrow SO_2 + O \qquad (7)$$

Rate constants for reactions (5)–(7) have not been determined experimentally. However, it seems reasonable that SH should be oxidised in the atmosphere to SO_2 by reactions (5)–(7) or by some other reaction mechanism. Measurements by Kurylo of the rate constant for reaction (4) and for the corresponding reaction with CS_2 are in conflict with results reported by Atkinson *et al.*[14]. Sources of the discrepancies are discussed in detail in ref. 6, and are attributed to complications due to photolysis of COS and to the occurrence of secondary reactions. Kurylo's experiments were performed in conditions designed to minimise these problems and his rate constants have been adopted accordingly. We estimate a global mean lifetime for COS of 200 days. Combining this lifetime with measured concentrations of COS[4,15], 0.5 ± 0.1 p.p.b. (parts per 10^9, by volume) we infer a global destruction rate for COS equivalent to 4 ± 0.8 Mtonnes S yr^{-1}.

Oxidation of CS_2 can provide a significant source of COS[6,7], through

$$OH + CS_2 \rightarrow SH + COS \qquad (8)$$

Subsequent oxidation of SH, through reactions (5) and (6),

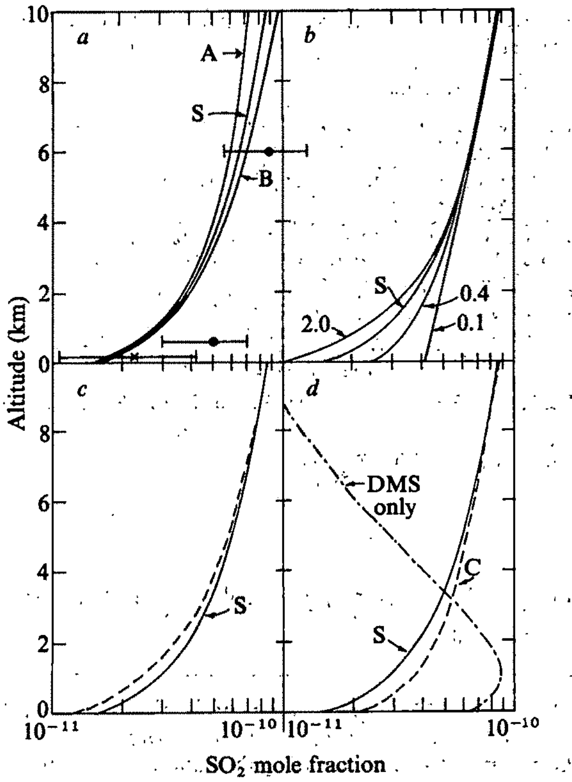

Fig. 1 Altitude profiles for OH and O_3 at 15° S latitude at equinox. *a*, Diurnally averaged concentrations for OH. The model calculations follow the time-dependent variation of short lived chemical species resulting from the diurnally varying radiation field which includes Rayleigh scattering. Calculations are shown for clear sky conditions (A) and a combination of clear sky (70%) and fully reflecting cloud layers at 3 km (17%) and 5 km (13%) (B). Vertical transport is simulated by 'eddy diffusion', with a diffusion coefficient equal to 10^5 cm^2 s^{-1}. The chemical model of Logan *et al.*[13] has been updated as described in Table 1. Fixed profiles for O_3[48] (see *b*), CO[49] (0.6 p.p.b.), CH_4[50] (1.5 p.p.b.) and H_2O (taken from the relative humidity values of Rasmusson[51]) were used. The mixing ratio of $NO+NO_2$ was taken to be 10^{-11} at 0 km, (ref. 52) and a deposition velocity of 1 cm s^{-1} was used for HNO_3. Note that the model implies a value of 1×10^6 molecules cm^{-3} for the globally averaged concentration of tropospheric OH.

should provide an additional source for SO_2. We estimate a lifetime for CS_2 due to reaction (8) of 60 days. Observational data for CS_2 are sparse, confined to the UK[15], and indicate a range of mixing ratios between 0.07 and 0.37 p.p.b. Sandalls and Penkett[15] point out that their data are consistent with the view that the ocean and atmosphere are in equilibrium with respect to CS_2. Using marine measurements of CS_2 by Lovelock[16], and solubility data by Seidell[17], they inferred an equilibrium concentration for the atmosphere equivalent to 0.22 ± 0.06 p.p.b. We adopt this value for the mixing ratio of CS_2 to represent global mean conditions. Used in conjunction with the chemical model of Table 1 and with the OH data of Fig. 1, it implies a global destruction rate for CS_2 equal to 12 ± 3 Mtonnes S yr^{-1}. The associated sources for COS and SO_2 are 6 ± 1.5 Mtonnes S yr^{-1} and 12 ± 3 Mtonnes S yr^{-1} respectively. Note that the source for COS due to oxidation of CS_2 could account for the bulk of atmospheric COS as discussed by Sze and Ko[7].

Source rates quoted here are directly proportional to values calculated for the concentration of OH. Studies of CH_3CCl_3[18,19] suggest that concentrations of OH given in Fig. 1 may be too high by about a factor of 2. If valid, this analysis would require corresponding reduction in source strengths inferred for CS_2 and COS. Relative abundances of CS_2, COS and SO_2 in remote locations are comparatively insensitive to details of the chemical model for OH, and provide a useful test of the hypothesis proposed here, that is, that oxidation of CS_2 and COS might represent dominant background sources of SO_2. Our model predicts concentration ratios CS_2:COS:SO_2 at 6 km, of 0.3:1.0:0.13 which may be compared with observed ratios[4] of 0.44:1.0:0.17. This is a fairly good agreement, when one remembers that the observational data for COS and SO_2 are uncertain to within about ±30%, that our value for the concentration of CS_2 was inferred from measurements of dissolved CS_2, and that the temperature dependencies of the rate constants for the key reactions (1), (4) and (8) are not well defined. In our model, one-half of the SO_2 source is derived from oxidation of CS_2, for which observational data are extremely limited. The mixing ratio for COS is fairly well defined and, given the uncertainties in the rate constants for reactions (1), (3) and (4), oxidation of COS alone might provide the major source for background SO_2.

Results and discussion

Calculated height profiles for SO_2 are shown in Fig. 2, which includes comparison with the observations by Maroulis *et al.*[4] and Nguyen Ba Cuong *et al.*[20]. We define a standard model, *S*, characterised by rate constants and parameters summarised in Table 1. Our choice of rate constant for reaction (1) is based on data reported by Castleman *et al.*[21] and Cox[22]. In their studies the rate for reaction (1) was measured relative to the rate for reaction of OH with CO. We applied suitable correction to account for the known pressure dependence[23] of OH+CO.

Our standard model assumes that the rate for reaction (1) is independent of temperature. Dependence of results on choice of rate expressions for reaction (1) is shown in Fig. 2*a*, which also includes models allowing modest negative activation energies

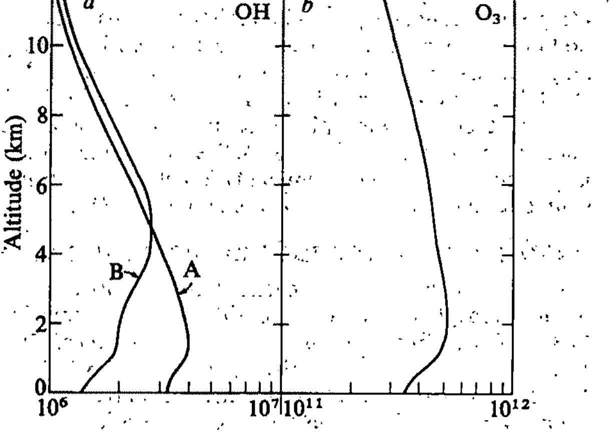

Fig. 2 Altitude profiles for SO_2 at 15° S latitude at equinox. The profiles were calculated with the model given in Fig. 1, curve B. The curves labelled S used the standard chemical model of Table 1, with a deposition velocity for SO_2 of 1 cm s^{-1} and a zero-flux boundary condition at the tropopause. The COS mixing ratio was specified at 0.5 p.p.b. Data are from Maroulis *et al.*[4] (●), aircraft flight over the Pacific Ocean, 0–67° S); and Nguyen Ba Cuong *et al.*[20] (x), Indian Ocean cruise, 5–25° S). *a*, The effect of temperature dependent rate constants: curve A, k_b $(OH+SO_2)=5.7\times10^{-33}$ exp (1,000/*T*); curve B, $k_4=3.4\times10^{-14}$ exp (150/*T*), $k_8=1.1\times10^{-13}$ exp (150/*T*). *b*, Uses the standard model *S*, but with values for the deposition velocity for SO_2 equal to 2.0, 0.4 and 0.1 cm s^{-1} as noted. *c*, The dashed curve includes loss of SO_2 to aerosols, with a sticking efficiency of 10^{-3}, as described in the text. *d*, The effect of a source of SO_2 from oxidation of DMS. Curve C includes an ocean source of DMS of 2.5 Mtonnes S yr^{-1}, in addition to SO_2 sources from CS_2 and COS oxidation, and may be considered as our best model. The curve labelled 'DMS only' omits all sources of SO_2 except that from DMS oxidation, and allows for a DMS source of 25 Mtonnes S yr^{-1}, the value suggested recently by Nguyen Ba Cuong *et al.*[37]. The calculated profiles of SO_2 exhibit small (<15%) variations with latitude between 45° N and 45° S.

for reactions (4) and (8). It seems probable that rates for reactions (4) and (8) should be faster at low temperature, given the reaction mechanism proposed by Kurylo[6,24]. The temperature dependence of k_1 is poorly determined. The rate expression given in Fig. 2*a* was based on preliminary work by Castleman and Tang[25] and Erler *et al.* (ref. 26 and results in ref. 10).

Figure 2*b* shows the dependence of calculated profiles for SO_2 on the choice of deposition velocity, while Fig. 2*c* shows the possible influence of heterogeneous loss due to aerosols. The general behaviour of the profiles in Fig. 2*a–c* is similar. In all cases we find higher mixing ratios at high altitude, consistent with the observational data of Georgii *et al.*[27] and Maroulis *et al.*[4]. Uncertainties in loss processes tend to affect mainly concentrations computed for the lowest altitudes. Deposition velocities over land[8] are reported in the range 0.1–2.0 cm s^{-1} while Hicks and Liss[28] suggest that a value of about 1 cm s^{-1} should be appropriate for moderate wind conditions at sea.

Results in Fig. 2*c* imply that loss of SO_2 to aerosols should be relatively unimportant, at least in the marine atmosphere. We used an integrated surface area for aerosols equivalent to 1×10^{-7} cm^2 per cm^3, an estimate based on particle size distributions measured by Meszaros and Vissy[29], Blifford[30], and Jaenicke *et al.*[31]. We investigated sticking efficiencies for SO_2 as high as 10^{-3}, although experimental data[32] suggest efficiencies much lower than this, in the range 10^{-3}–10^{-6}. Results in Fig. 2 do not allow for transient reductions in SO_2 which might arise due to rainfall.

We assumed a rate constant for reaction (3) equal to 10^{-15} $\text{cm}^3\ \text{s}^{-1}$. With this choice, reaction (3) represents a relatively minor sink for SO_2. Measurements by Kan *et al.*[33] indicate removal of CH_3O_2 by SO_2, with an effective rate constant of $5\text{–}10 \times 10^{-15}$ $\text{cm}^3\ \text{s}^{-1}$. If loss of CH_3O_2 observed in the laboratory is associated with the reaction channel (3), concentrations in Fig. 2 could be reduced by about 20%. This estimate assumes, however, an unproven model for oxidation of atmospheric CH_4. It seems unlikely that k_3 should be as large as 10^{-14} $\text{cm}^3\ \text{s}^{-1}$ given the much smaller value, $\leq 10^{-17}$ $\text{cm}^3\ \text{s}^{-1}$ (refs 34, 58), reported for the analogous reaction (2).

Oxidation of DMS could provide an additional source of SO_2 as suggested by Lovelock[35] and discussed further by Maroulis and Bandy[36] and Nguyen Ba Cuong *et al.*[37]. The associated source of SO_2 would be confined to the lowest 2 km of the atmosphere as the reaction of DMS with OH is exceedingly rapid[38]. Thus DMS cannot account for the height profile observed for SO_2, a point shown in Fig. 2*d*. Similar arguments apply to the suggestion that oxidation of H_2S might produce a large source of SO_2. Hydrogen sulphide also reacts very rapidly with OH[39], and concentrations as low as a few parts per trillion have been observed in tropical marine air[40]. Most estimates of the contribution of H_2S to the atmospheric sulphur cycle have been derived by subtracting other terms in the budget for sulphur, and are consequently highly uncertain[1,2]. While biogenic sources of H_2S and DMS may provide significant contributions to the atmospheric sulphur cycle in selected environments (for example, near swamps or salt marshes), these sources cannot account for the observations of SO_2 discussed here.

Liss and Slater[41], using measurements by Lovelock *et al.*[35], estimate a net source of 3.7 Mtonnes S yr^{-1} associated with release of marine DMS, a source whose magnitude should be reduced by about a factor of 1.5 to account for more recent estimates of rates for gas exchange across the air–sea interface[42].

It seems probable that the concentrations of atmospheric CS_2 and COS should reflect a combination of biogenic and industrial sources[43,44]. Preliminary measurements of the emission rates of sulphurous gases over salt marshes have shown that fluxes of COS might be comparable to, and in some cases greater than fluxes of H_2S[45]. The relatively uniform distribution[4,13,46,47] observed for COS suggests a globally distributed source. This apparently argues against a significant industrial component, although there might be important production due to forest fires and combustion associated with land clearance for agriculture. The possibility of a dominant industrial component for CS_2 cannot be excluded, given limitations in the present data base. It seems more likely, however, that diffuse sources should dominate for CS_2 as well as for COS. These sources may include a component due to combustion although thermodynamic considerations suggest that reactions in reducing environments may be more important. It seems probable that these reactions should be microbially mediated: swamps, anaerobic muds and the digestive tracts of various animals offer plausible sites. The relatively uniform distribution observed for SO_2 in the upper marine troposphere[4] requires a diffuse atmospheric source for

Table 1 Kinetic data

No.	Reaction	Rate expression*	Refs and notes
1	$OH + SO_2 + M \rightarrow HSO_3 + M$	$k_a = 6.8 \times 10^{-13}$ $k_b = 1.6 \times 10^{-31}$ $1/k = 1/k_a + 1/k_bM$ $k_1 = k \times (k_9/1.4 \times 10^{-13})$	Fit to the data of refs 21, 22
2	$HO_2 + SO_2 \rightarrow SO_3 + OH$	2.0×10^{-17}	34
3	$CH_3OO + SO_2 \rightarrow$ products	1.0×10^{-15}	See text
4	$OH + COS \rightarrow SH + CO_2$	5.66×10^{-14}	6
7	$OH + CS_2 \rightarrow SH + COS$	1.85×10^{-13}	6
8	$OH + (CH_3)_2S \rightarrow$ products	$6.08 \times 10^{-12} \exp(134/T)$	38
9	$OH + CO \rightarrow CO_2 + H$	$2.10 \times 10^{-13} f(M) \exp(-115/T)$ $f(M) = (1 + 3.7 \times 10^{-20} \times M)$	53 23
10	$HO_2 + O_3 \rightarrow OH + 2O_2$	$1.40 \times 10^{-14} \exp(-580/T)$	Zahniser and Howard (personal communication)
11	$HO_2 + NO \rightarrow OH + NO_2$	$3.3 \times 10^{-12} \exp(254/T)$	54
12	$HO_2 + HO_2 \rightarrow H_2O_2 + O_2$	$3.9 \times 10^{-14} f(H_2O) \exp(1245/T)$ $f(H_2O) = 1 + 2.5 \times 10^{-18} \times [H_2O]$	55, 56
13	$H_2O_2 + h\nu \rightarrow OH + OH$		57
14	$CH_3OO + NO \rightarrow CH_3O + NO_2$	8.0×10^{-12}	Estimated
15	$CH_3OO + HO_2 \rightarrow CH_3OOH + O_2$	3.0×10^{-12}	Estimated
16	H_2O_2 heterogeneous loss (units, s^{-1})	1.16×10^{-5} 0–4 km $1.16 \times 10^{-5} \exp(1.6 - 0.4\,z)$ 4–12 km	
17	SO_2 + aerosol $\rightarrow$ heterogeneous loss (probability)	0.0	†

* Units $\text{cm}^3\ \text{s}^{-1}$ for bimolecular reactions; $\text{cm}^6\ \text{s}^{-1}$ for termolecular reactions. The complete chemical scheme is described in Logan *et al.*[13].

† The integrated surface area for marine aerosols was taken to be 1.0×10^{-7} cm^2 per cm^3, and a scale height for the aerosol distribution function of 1 km was adopted (see refs 29–31).

this gas. Oxidation of COS and perhaps CS$_2$ as discussed here can provide a relatively straightforward explanation for the existence of a uniform background level of SO$_2$.

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The effects of past variations of the Earth's rotation rate on climate

B. G. Hunt

Australian Numerical Meteorology Research Centre, PO Box 5089AA, Melbourne, Australia 3001

Palaeological evidence indicates that the rotation rate of the Earth during the late Precambrian was 2–2.5 times faster than now. Typically high rotation rate in a fluid system reduces the characteristic size of dynamic features, their associated transport processes and the overall intensity of the motions. The climatic consequences of the high rotation rate during the Precambrian are outlined. In particular, it is hypothesised that variations in the rotation rate imposed an important, and possibly dominant, effect on the genesis and termination of the Precambrian ice age. Other possible implications of the high rotation rate to oceanography, life forms and geology are briefly noted.

POTENTIAL mechanisms for producing climatic variations include solar perturbations owing to orbital variations of the Earth[1], volcanic activity[2], tectonic movements[3], fluctuations in solar output[4], glacier surgings[5] and oceanic feedback[6]. On a time scale of hundreds of millions of years a further factor, which has received relatively little attention[7], is the increase in the rotational period of the Earth. This increase is very small ($\sim$2.5 ms per century) and is still taking place. However, since the atmospheric and oceanic circulations are known to be critically influenced by the Earth's rotation rate it follows that climate should also be affected. Hence it is of value to consider what impact past variations in the rotation rate might have had on the evolution of the Earth's palaeoclimate, and to see how well the deductions fit the known facts.

This assessment will be obtained by interpreting the results of a numerical model of the atmospheric circulation. This general circulation model of the atmosphere was hemispheric in extent, devoid of topography and thus oceanic influences, and was set up for fixed annual mean conditions[8]. Despite these apparently serious omissions the model is capable of a remarkable simulation of many features of the atmosphere[8,9], which encourages the belief that its application to problems concerned with variations in the rotation rate of the Earth has merit. This model has now also been run with the rotation rate increased and decreased by a factor of 5 as part of a spearate study[10], and it is this data base of three experiments which will be used here.

Prehistorical perspective

The rotational history of the Earth can be traced back to the late Precambrian era, about 1.5×10^9 yr ago. Based on growth patterns from shells and corals the Earth then had at least 800–900 solar days per yr, implying a rotation rate 2–2.5 times greater than the present value[11]. MacDonald [12] has calculated the past variation of the rotation rate and his results for one set of assumptions are shown in Fig. 1. This illustrates a possible increase in rotation rate with decreasing time up to about

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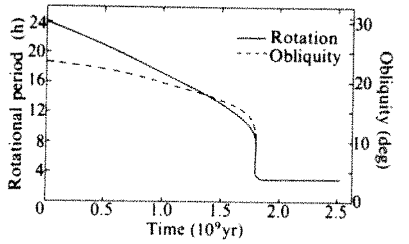

Fig. 1 Variation with time of Earth's rotational period (left-hand scale) and obliquity (right-hand scale).

1.7×10^9 yr ago, when a discontinuity associated with the closest approach of the Moon to the Earth may have occurred. Such a discontinuity, if real, would have created catastrophic climatic and other changes. Consequently the emphasis here will be placed on the less dramatic, but continuous, climatic effects associated with the subsequent steady decrease of the rotation rate between 1.7×10^9 yr ago and the present.

It is also necessary to consider other factors which may have been important to climatic evolution in this time frame in order to clarify the contribution from rotation rate variations. Thus continental drift, as recently reconstructed by Irving [13] back to the middle Devonian (375×10^6 yr ago), could markedly affect climate. This can occur via continentality effects associated with large land masses[14], or through changes to ocean depths or basin sizes[15]. In particular shallower oceans would have had a less ameliorating influence on climatic developments. (Note that rotation rate variations would also have affected the dynamics of the motions in the molten interior of the Earth, and therefore geomagnetic activity, in addition to the motions of the atmosphere and oceans. Presumably such dynamic variations could have influenced the large-scale convective motions responsible for tectonic activity and volcanism, and this could be an important factor in the geological evolution of the Earth.) A further prehistorical factor is the change in the obliquity of the Earth's Equator to the ecliptic associated with the dynamics of the Earth–Moon system (Fig. 1). The lower obliquity in the past implies less annual mean solar input to high latitudes and therefore possibly colder polar regions, all else being the same. The luminosity of the Sun has also probably increased with time; a 25% increase over the past 4.5×10^9 years has been suggested[16]. Wetherald and Manabe[17] have shown that 1–2% variations in the solar constant could have significant effects on the climate of a general circulation model, particularly at high latitudes. Composition changes in the atmosphere during its evolution are also relevant, and contribute to a computed 10 K increase in mean surface temperature over the past 1.5×10^9 yr (ref. 16). The principal change in composition during this time span is supposedly in the O_2/N_2 ratio. A further important factor in climatic variability is volcanic activity[2]. Other forcing functions could be enumerated but not all of these are relevant to the time frame of interest here. The above list is thought to contain the principal precursors of climatic change which should be considered simultaneously with an evaluation of the contribution associated with the decrease in the Earth's rotation rate.

Model climatology at high rotation rate

A summary of the results of the general circulation model run with a fivefold increase in rotation rate[10] is given here to illustrate the markedly different atmospheric behaviour in these conditions. For simplicity this model was run with all radiative terms (including cloud cover, surface albedo and CO_2 amount) set at current values. These and other model parameterisations are based on present atmospheric conditions which would not necessarily apply in the extreme conditions considered here. This undoubtedly distorted the details of the results but the dynamically induced changes in the large scale circulation are reasonably realistic.

An important difference is shown in Fig. 2, where a comparison of near surface (0.9 km) synoptic meridional velocity distributions for this model and the control (normal rotation rate) reveals a distinct reduction in scale size at high rotation rate. Wavenumber 13 predominates compared to wavenumber 6 for the control. This result is not resolution limited[18], and in fact the overall behaviour of the model at all three rotation rates closely resembled the general flow characteristics produced by laboratory analogues of the atmosphere in appropriate conditions[19,20]. A consequence of the smaller scale size was that the synoptic distributions of the various atmospheric fields (velocity, temperature, moisture) were poorly correlated. This resulted in a reduced tropospheric polewards transport of sensible and

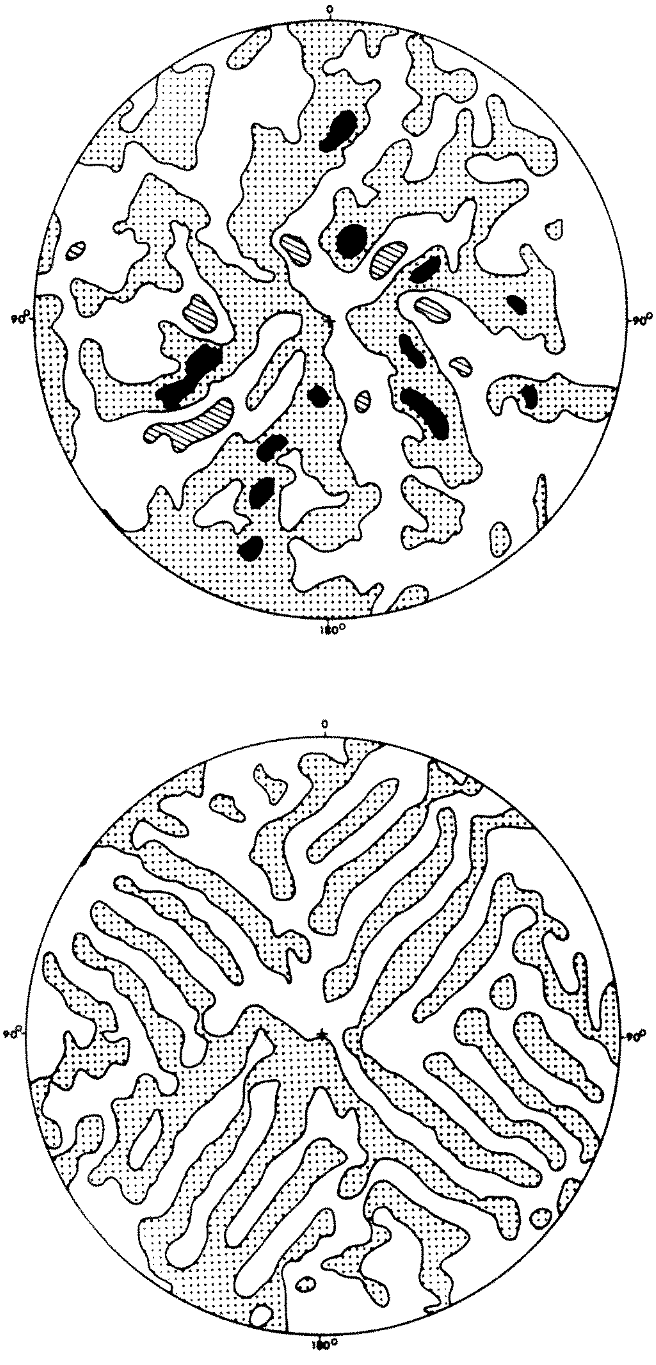

Fig. 2 Instantaneous synoptic meridional (north–south) velocity distributions for normal rotation and fivefold rotation rates are given in top and bottom of figure respectively. The circumscribing circle is the Equator, the + sign the Pole. Solid areas, $>10\ \mathrm{m\ s^{-1}}$; stippled, $0\text{–}10\ \mathrm{m\ s^{-1}}$; blank, $0\text{–}(-10)\ \mathrm{m\ s^{-1}}$; cross-hatched, $>-10\ \mathrm{m\ s^{-1}}$.

latent heat by the dominant large-scale eddies, thus causing a much colder high latitude region and a warmer tropics.

The mean meridional stream function increased from three to five cells, with the tropical Hadley cell being markedly reduced in latitudinal extent and intensity. The mean zonal winds were almost halved in intensity, with the tropospheric westerly jet stream being located at 10° latitude. The stratospheric westerly jet virtually ceased to exist as a separate entity. A simple application of the thermal wind equation suggests that a more intense tropospheric jet should have been obtained because of the increased tropospheric latitudinal temperature gradient in the model. However, this equation is invalid at fast rotation rates where motions are highly ageostrophic, and again the model's behaviour is confirmed by laboratory analogue experiments[19,20].

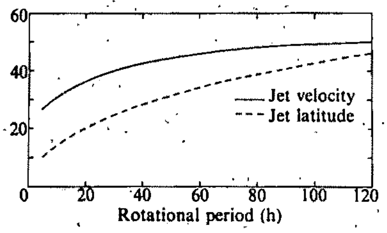

Fig. 3 Variation with Earth's rotational period of the intensity of the mean zonal wind ($m\,s^{-1}$) of the tropospheric jet, and of the location of the jet (deg latitude).

The conventionally defined arid region of the globe narrowed in latitudinal extent and moved towards the Equator, but most of the globe polewards of 45° also became semi-arid as the increased coldness of the troposphere caused a general decline in evaporation and precipitation in this region.

Implications of rotation rate variations for Earth's climate

Consider first the overall impact of the change in wind strength with rotation rate as derived from the three model experiments. This is illustrated in Fig. 3 by the variation of the intensity and latitude of the tropospheric jet stream, which is located at a height of about 10 km. This indicates that for a rotational period of 9 h (late Precambrian era) the jet stream intensity was reduced by about 20% compared to its current value, but, more importantly, the jet was located much closer to the Equator at 15° latitude. As illustrated in Fig. 1 of ref. 10 the confinement of the jet to low latitudes was associated with a reduction in the overall intensity of the zonal winds in the troposphere as a whole, to a much greater extent than that implied by the 20% reduction in jet intensity. A prime consequence of this constraint is that in Precambrian times the surface wind stress, which is normally proportional to the square of the wind velocity, would have been reduced by possibly as much as a factor of 2. A rough indication of this change can be obtained by comparing the intensity and variability of the near surface synoptic wind distributions in Fig. 2.

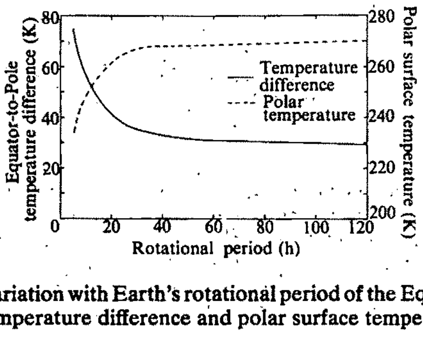

Fig. 4 Variation with Earth's rotational period of the Equator-to-Pole temperature difference and polar surface temperature.

Perhaps the single most important effect of the reduced surface stress would have been its impact on the oceans. For example there would have been less wind-induced vertical mixing in the ocean, resulting in a shallower, warmer mixed layer, in general, with presumably a smaller overall heat capacity. A smaller heat capacity would make the oceans less able to reduce the amplitude of seasonal and interannual fluctuations of ocean sea surface temperature, which are important in determining the behaviour of the atmosphere[21]. In addition the scales of motion in the ocean would have been reduced by the higher rotation rate of the Earth, which, together with the lower stress, should have weakened the strength of the oceanic gyres. This would also have decreased the amount of heat transported polewards by the oceans, the latter being a significant component of the total polewards flux of energy[22]. The net effect of all these changes should be to produce a general warming of the tropical oceans and atmosphere and a colder polar region, possibly associated with greater transient departures from mean conditions. The smaller obliquity of the Earth (Fig. 1) might also have complemented these rotationally induced variations.

An additional oceanographic effect to be expected under Precambrian rotational conditions is a reduction in upwelling (depending on continental configurations) and therefore in the supply of nutrients to surface layers. This would have discouraged biological activity and may have some implications to metazoan evolution[23].

All of the above comments should be qualified by noting the tremendous changes which have occurred in oceanic basins during geological history[13]. Some of these changes may well have ameliorated the purely rotational aspects considered here.

A further aspect of the lower wind stress at higher rotation rate concerns atmospheric composition. Transfer of gases between atmosphere and surface would have been restricted, as would, presumably, vertical transport in the troposphere. In addition, the enhanced coldness and aridity at high latitudes would have reduced biological activity, which is crucial to the production of minor, but important, atmospheric constituents such as N_2O, NH_3 and CH_4. Such subtle effects may have influenced the overall development of atmospheric composition, notably that of O_3 which is considered to be a vital constituent in the development of life on land because of its UV screening efficiency[24].

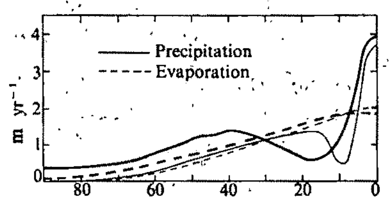

Fig. 5. Comparison of time averaged zonal mean precipitation and evaporation rates for normal rotation (thick lines) and fivefold rotation rates (thin lines).

The net consequence of the reduced polewards heat fluxes suggested by the model for Precambrian times is a colder polar region and an enhanced Equator-to-Pole temperature difference as shown in Fig. 4. A 20 K colder pole is indicated for a rotational period of 9 h, unless the polar cloud cover and thickness were different. The latter may well have been the case given the reduced water vapour content of the colder atmosphere and the smaller-scale systems. Certainly Hart's[16] global mean calculations suggest an overall cloud cover of only ~30% at this time in conjunction with a mean surface temperature of 280 K. This temperature is over 10 K colder than that of the fast rotation rate general circulation model, perhaps indicating that simple comparisons between the two approaches are not meaningful.

Although the model implies that the precipitation rate was very small at high latitudes during the Precambrian era (see Fig. 5), conditions should have been conducive to the accumulation of ice cover, depending on the dispositions of the continent(s?)[14]. On this basis it can be hypothesised that glacial conditions prevailed throughout the Precambrian, and solely because of rotational considerations! The extent of the ice cover would have declined continuously, all else being equal, as the Earth's rotation rate decreased and the improved circulation systems warmed high latitudes. This explanation can also reasonably account for the termination of the Precambrian ice age 6×10^8 yr ago, when the rotational period of the Earth would have been 20 h and thus dynamical conditions less conducive to the maintenance of glaciation. The occurrence of the Permo-Carboniferous ice age ($\sim3\times10^8$ yr ago) and the present Quaternary ice age are somewhat paradoxical, especially when the assumed increase of the solar output[16] is taken into consideration. Continental drift, volcanic activity and so on may play some part but other unknown factors seem to be important. For example, energy budget studies indicate that in present conditions an ice-free Arctic Ocean would be self-sustaining[25], thus raising the problem of the initial cause of the glaciation.

In Fig. 5 the zonal mean evaporation and precipitation rates for the fast and control models are compared. Interpolating results to Precambrian conditions indicates that equatorial conditions were similar to those of today but that the adjacent zone of high aridity was narrower and closer to the Equator. The general aridity and/or coldness at higher latitudes implied by Figs 4 and 5 suggests a rather harsh climate unattractive to all but the hardiest life-forms at that time. Higher latitudes would have become progressively habitable as the Earth's rotation rate slowed, but the subtropical very arid zone would have expanded. Because of the smaller-scale and less organised nature of meteorological systems suggested for the Precambrian, precipitation generally would have been less intense and more localised. This means that run-off and erosion would have been lower and this could have some relevance to the interpretation of, say, alluvial deposits.

Although the above discussion of Precambrian conditions is necessarily speculative, certain features can be viewed with some confidence. Among these are the delineation of climatic zones in Fig. 5, and the jet stream variations of Fig. 3, both of which are closely associated with the requirements for conservation of absolute angular momentum in the Earth–atmosphere system. Similarly the small scale size of dynamical features is a well established characteristic of high rotation rate[19,20]. Thus, although distortion of the model results could possibly have arisen from inadequacies of the radiative scheme used, grossly different results should not be expected.

Finally, since a general appreciation of the past variation of the Earth's rotation rate appears to be lacking, it might be useful to reinterpret data from several other disciplines where rotational effects could prove to be of importance.

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Single strands induce recA protein to unwind duplex DNA for homologous pairing

Richard P. Cunningham, Takehiko Shibata, Chanchal DasGupta & Charles M. Radding

The Departments of Internal Medicine, and Molecular Biophysics and Biochemistry, Yale University School of Medicine, New Haven, Connecticut 06510

Single-stranded DNA, whether homologous or not, stimulates purified Escherichia coli *recA protein to unwind duplex DNA. This helps to explain how recA promotes a search for homology in genetic recombination. As oligodeoxynucleotides also stimulate unwinding, a common mechanism may relate the function of recA protein in recombination to other functions (SOS) induced by oligonucleotides.*

BECAUSE breakage of DNA by various means stimulates general genetic recombination, some investigators have suggested that recombination begins with the interaction of a single strand with duplex DNA (see ref. 1 for review). As a model for this kind of interaction, Holloman *et al.*[2] and Beattie *et al.*[3] studied the uptake of homologous single strands by superhelical DNA [replicative form I (RFI) or form I], a reaction that produces a D-loop (see Fig. 1). By transfection of *Escherichia coli*, Holloman and C.M.R.[5] implicated the *recA* gene in the homologous interaction of a single-stranded fragment with superhelical DNA. More recently, Shibata *et al.*[4] observed that stoichiometric amounts of highly purified recA protein promoted the rapid formation of D-loops by superhelical DNA and homologous single-stranded fragments (Fig. 1).

The properties of the uncatalysed formation of D-loops, which occurred rapidly only at non-physiological temperatures,

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suggested that the rate was limited by the initial unstacking of a small number of base pairs in superhelical DNA[3]. As recA protein bypassed this rate-limiting step, we suggested that it acted in part by unstacking base pairs in duplex DNA. We also observed that a preparation of nicked circular DNA (form II) was one-third as active as superhelical DNA in forming complexes with single-stranded fragments[4]. Subsequently, we have confirmed that form II DNA is a substrate for the formation of D-loops by recA protein (see below). The latter observation strengthened the inference that recA protein has an unstacking or unwinding activity, which is the subject of this article.

Unwinding of duplex DNA by recA protein in the presence of ATPγS and single strands

To obtain direct evidence that recA protein unwinds DNA, we incubated it with fd form II DNA, added DNA ligase, and examined the product by gel electrophoresis and isopycnic centrifugation in CsCl plus propidium diiodide[6,7].

In the absence of recA protein, ligase sealed more than half of the form II DNA as indicated by gel electrophoresis (Fig. 2*a*, *b*) and by the assay of Kuhnlein *et al.*[8] for covalently closed DNA. At the ionic strength of the gel, these covalently closed molecules (form I') migrated as a set of bands of positively superhelical DNA, which overlapped a band due to linear DNA (form III) present in the preparation (Fig. 2*b*). When recA protein and adenosine 5'-*O*-(3-thiotriphosphate) (ATPγS) were incubated with form II DNA before ligation, the observed distribution of products was similar; ligase worked well but there was no apparent change in the average linking number of the population of sealed molecules (Fig. 2*c*). However, when we added fragments of single-stranded ΦX174 DNA during the incubation with recA protein, we observed a very different result. Ligase still closed about one-third to one-half of the fd form II DNA, but all the closed DNA migrated as a single band of more compact DNA, at about the position of form I (Fig. 2*d*). In three experiments, when recA protein and ATPγS were incubated with form II DNA in the absence of any added single-stranded DNA before ligation, we observed the formation of a small amount of material with the electrophoretic mobility of form I (see Table 1, lines 1 and 2). Controls included the following variations of the mixture represented in Fig. 2*d*: no recA protein; no form II DNA; no ATPγS; ATP in place of ATPγS; recA protein in the absence of ligase (Fig. 2*a*). In no case was DNA detected in the position of the rapidly migrating band.

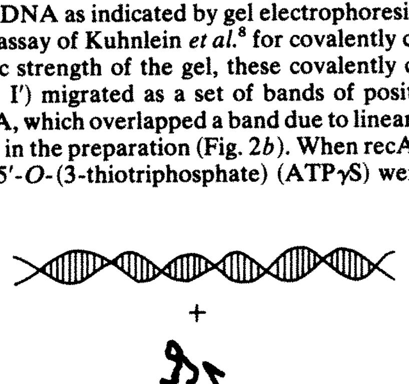

Fig. 1 The formation of a D-loop catalysed by recA protein[4]. The actions of recA protein apparently include (1) unwinding double-stranded DNA, (2) unfolding single-stranded DNA, (3) homologous pairing, and (4) uptake of a single strand rather than rewinding of the original duplex DNA.

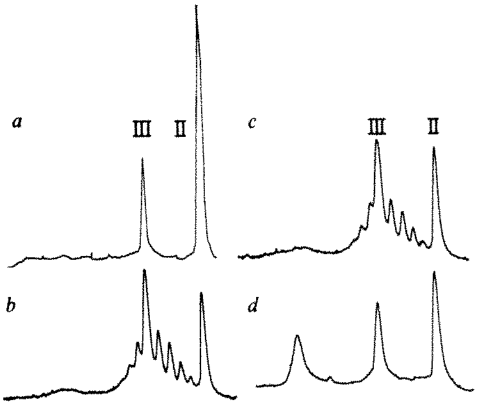

Fig. 2 The linking number of circular DNA sealed in the presence of recA protein and ATPγS (Boehringer-Mannheim). Microdensitometer tracings of electrophoretic gels prepared as described in Fig. 4, legend. *a*, Control, fd form II DNA incubated with recA protein but no ligase. The preparation of DNA contained about 30% form III DNA as indicated. *b*, Ligase only (New England BioLabs). *c*, Ligase plus recA protein. *d*, Ligase, recA protein and 12-μM fragments of heterologous single-stranded DNA from ΦX174. The various forms of circular DNA from phages ΦX174 and fd were prepared as cited previously[4]. Single-stranded fragments were prepared by boiling 840 μM DNA for 15 min in 0.1 mM EDTA and 10 mM Tris-HCl at *p*H 7.5. All concentrations of DNA are expressed as mol of nucleotide. The reaction mixture was the same as that described in Table 2 legend, except that heterologous single-stranded fragments were substituted for homologous fragments, the volume was doubled and ATP was replaced by 0.5 mM ATPγS. After incubation at 37 °C for 30 min, the mixture was chilled on ice, KCl, $(NH_4)_2SO_4$ and NAD^+ added to make their concentrations 25 mM, 10 mM, and 25 μM, respectively, and incubated at 18 °C for 5 min. 0.3 units of *E. coli* DNA ligase were then added and the sample, 50 μl total volume, incubated for 90 min. The reaction was stopped by adding EDTA, 28 mM, and SDS, 0.17%. The sample was transferred to a bath at 37 °C, proteinase K, 0.2 mg ml^{-1} added and the sample incubated for 15 more minutes.

To determine whether the compact closed DNA had a lower or a higher linking number than the DNA closed by ligase alone (form I'), we centrifuged the products of a similar experiment in an isopycnic gradient of CsCl and propidium diiodide (Fig. 3 and Table 1). In the absence of recA protein, half of the recovered DNA was found in a single peak at the dense end of the gradient, as expected for form I' DNA derived from the closure of form II by ligase (Fig. 3*b*, Table 1, line 1). Incubation of form II DNA with recA protein and ATPγS before ligation had little effect on the distribution (Fig. 3*a*, Table 1, line 2). However, when recA protein, fd form II DNA, ΦX174 single-stranded fragments and ATPγS were incubated before ligation, the peak of form I' DNA at the dense end of the gradient disappeared almost entirely and was replaced by DNA with a broad distribution of densities

Table 1 Unwinding of DNA

		Distribution (%)		
	Total recovery (%)	I'	Intermediate	II+III
Ligase only	22	46	4	50
+recA protein	29	39	7	54
+recA protein and fragments	28	8	21	71

Data tabulated from Fig. 3. The DNA labelled 'Intermediate' has a lower linking number than form I' DNA, the product of sealing by ligase alone.

between those of form I' and linear or form II DNA (Fig. 3*a*, Table 1, line 3), that is, DNA with lower linking numbers than form I'. The peak of material of intermediate density corresponded to the position of negatively superhelical fd DNA (form I DNA). The total recovery of radioactivity was similar in the three parts of this experiment; the fraction of closed circular DNA relative to linear or form II DNA (Fig. 3*a*, Table 1, line 1) was the same as that estimated by the assay of Kuhnlein *et al.*[8], which indicates that the distribution of recovered material was not biased.

Lack of topoisomerase activity of recA protein

The unwinding observed in the experiments described above could have occurred before or after ligation, but three experiments show that recA protein lacks detectable topoisomerase activity in the presence of either ATPγS or ATP. We incubated recA protein with relaxed closed circular DNA (form I') in conditions identical to those of the experiments shown in Figs 2 and 3, including a sample which contained heterologous fragments and ATPγS. When we observed the product by gel electrophoresis, we found no DNA in the position of form I DNA, and no perceptible shift in the distribution of form I' DNA (Fig. 4). With the following additions or omissions, we examined, by gel electrophoresis, the fate of fd RFI incubated for 30 min at 37 °C with recA protein and fragments of single-stranded ΦX174 DNA: ATP; ATPγS; ATPγS without single-stranded fragments; ATPγS without recA protein. We observed little or no change in the amount of form I DNA (data not shown).

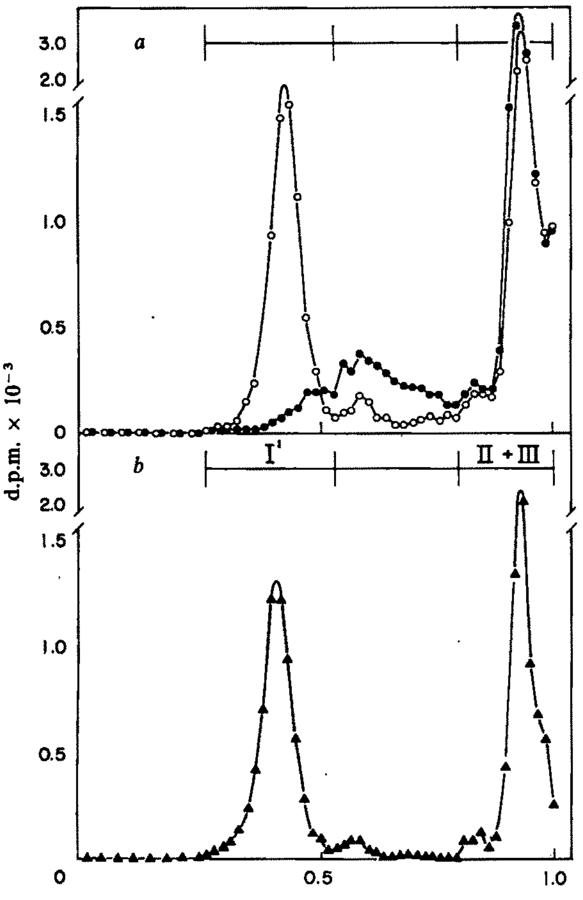

Fig. 3 Decreased linking number of circular DNA sealed in the presence of recA protein and ATPγS; isopycnic centrifugation in CsCl and propidium diiodide[7]. *a*, fd form II DNA sealed in the presence of recA protein only (○), and in the presence of recA protein plus heterologous fragments of single-stranded DNA from ΦX174 (●). *b*, Control. DNA sealed in the absence of recA protein (▲). The left end of the figure corresponds to the dense end of the gradient. We summed values as indicated by the brackets (Table 1). All values were corrected for quenching. Solutions, 7.4 ml, contained CsCl to make the density 1.565 g cm^{-3}, 500 μg ml^{-1} propidium diiodide, 10 mM Tris-HCl and 1 mM EDTA at *p*H 8.0. These solutions were centrifuged at 44,000 r.p.m. and 20 °C for 69 h in a Beckman 75ti rotor. The resulting gradients were collected from the bottom of the tube and counted in a scintillation fluor containing Triton X-100.

In further experiments we made D-loops by incubating recA protein with fd form I DNA and fragments of fd single strands in the presence of ATP[4]. Measurement by electron microscopy of 22 D-loops with one or two single-stranded tails showed that the mean length was 416±45 nucleotides. (We were unable to distinguish the double-stranded arm of the D-loop from the single-stranded one. As there was little difference in the lengths of the two arms of any D-loop, we simply measured both and calculated the average of all values.) In the conditions of the reaction, fd form I DNA should contain about 40 superhelical turns which corresponds to a D-loop about 400 nucleotides long. Previous observations have shown a rough equivalence between the length of D-loops measured by electron microscopy and the length predicted from the superhelix density in solution[9]. Observation of the same relationship in these experiments suggests that when recA protein catalysed the formation of D-loops from form I DNA, the linking number of the closed circular DNA was not markedly changed.

Table 2 Homologous pairing of double-stranded DNA and single-stranded fragments

Fragments	% fd double-stranded [^{3}H]DNA retained	
	form I	form II
None	1	4
fd	34	22
ΦX	2	6

The reaction mixture, 20.5 μl in volume, contained 31 mM Tris-HCl at *p*H 7.5, 25 mM $MgCl_2$, 1.8 mM dithiothreitol, 88 μg ml^{-1} bovine serum albumin, 1.3 mM ATP, 8.8 μM RF[^{3}H]DNA, 12 μM single-stranded DNA fragments and 98 μg ml^{-1} recA protein[4]. The mixture was incubated at 37 °C for 30 min, chilled and 18 μl transferred to 0.5 ml of cold 25 mM EDTA. Aliquots were taken from this mixture to measure total counts, D-loops and closed circular DNA, exactly as described before[4].

Nature of the cofactor for unwinding by recA protein

Unwinding is supported either by heterologous or homologous single-stranded fragments (Fig. 4*a*, *b*). Circular single-stranded DNA, which does not form D-loops, promoted unwinding (Fig. 4*d*). Double-stranded restriction fragments were inactive, but their boiled products were active (Fig. 4*f*, *g*). This observation indicates that the cofactor was not some fortuitous ingredient of the DNA preparations. A digest, consisting entirely of acid-soluble material produced by treatment with pancreatic DNase and boiling, also supported the unwinding activity very well (Fig. 4*e*). The following, added singly, promoted little or no unwinding: deoxyadenosine, dAMP, dGMP, dCMP, dTMP, AMP and CMP (data not shown).

Homologous pairing of form II DNA and single-stranded fragments

Table 2 compares form II and form I DNA as substrates for recA protein in the homologous pairing with single-stranded

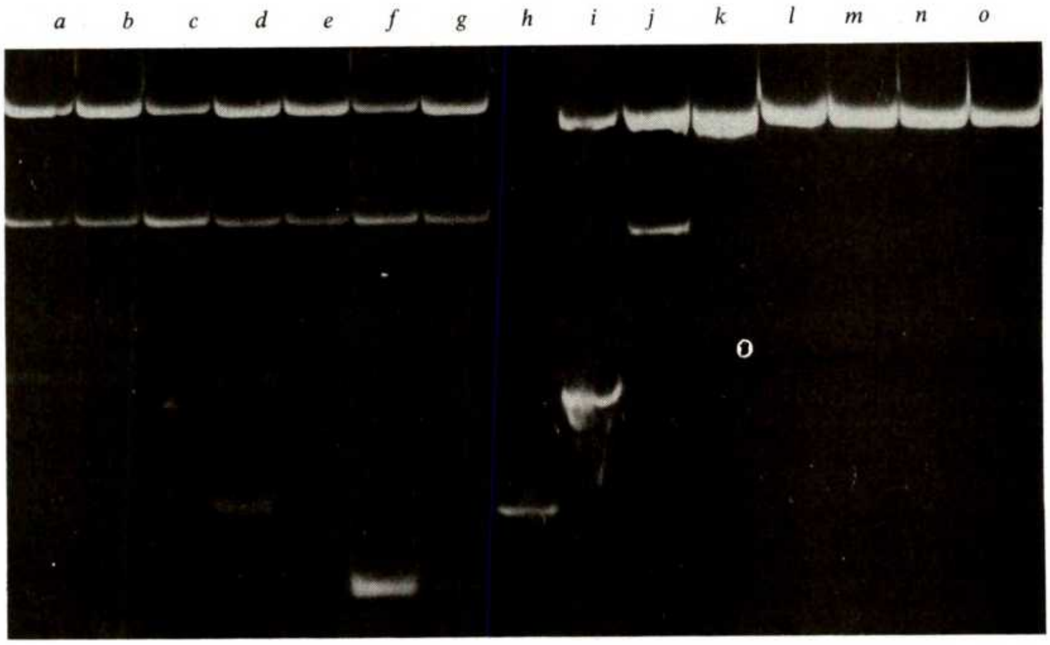

Fig. 4 Nature of the cofactor that stimulates unwinding by recA protein in the presence of ATPγS (*a–g*); absence of topoisomerase activity in recA protein (*l–o*): agarose gel electrophoresis. Lanes *a–g* contained fd form II DNA sealed in the presence of recA protein, ATPγS and 12 μM DNA cofactors: *a*, fragments of single-stranded ΦX174 DNA; *b* fragments of single-stranded fd DNA; *c*, no addition; *d*, fd phage DNA; *e*, boiled DNase I limit digest of ΦX174 RFI DNA; *f*, restriction endonuclease *Hae*III digest of fd RFI; *g*, the preceding digest boiled. Lanes *h–k* contained markers: *h*, fd phage DNA; *i*, fd RFI DNA; *j*, fd form II DNA (~70%) and fd form III DNA (~30%), *k*, fd form I' DNA. Lanes *l–o* contained fd form I' DNA incubated with recA protein and ATPγS: *l*, no addition; *m*, fragments of single-stranded ΦX174 DNA; *n*, preceding sample minus recA protein; *o*, fragments of single-stranded ΦX174 DNA plus ATP instead of ATPγS. To obtain the limit digest indicated above 24 μM ΦX174 RF DNA was treated with 20 μg ml^{-1} pancreatic DNase (Worthington) for 30 min at 37 °C in 5 mM $MgCl_2$ and 10 mM Tris-HCl at *p*H 7.5, which rendered all the DNA acid soluble. The product was boiled for 4 min to inactivate the pancreatic DNase and denature all the fragments. Nicked circular DNA, sealed by polynucleotide ligase was slightly positively superhelical under the conditions of gel electrophoresis (for example lane *c*) whereas closed circular DNA relaxed by calf thymus topoisomerase had a titrable superhelix density close to zero (for example lane *k*). We used 1% agarose gels, 16 cm × 16 cm × 0.3 cm in E buffer, which contained 40 mM Tris-acetate, 5 mM sodium acetate and 1 mM EDTA at *p*H 8.0. Current was applied at 35 V for 16–18 h, and the buffer was recirculated between the reservoirs, following which we stained the gels for 2 h in E buffer containing 0.5 μg ml^{-1} ethidium bromide. We illuminated the gels from below with a short-wavelength UV lamp (Ultra-Violet Products) and photographed them with Polaroid Tyffe 55 Land film. The negatives were scanned with a Joyce–Loebl recording micro-densitometer.

fragments. In the conditions of these experiments (see Table 2, legend), which differ slightly from previously described conditions[4], the preparation of form II DNA was one-half to two-thirds as active as form I DNA in making complexes that were retained by nitrocellulose filters. As the preparation of form II DNA contained less than a few per cent of form I DNA, these complexes must have been made with DNA that was not superhelical. The synthesis of complexes with form II DNA required homologous single-stranded fragments, and the properties of the product were similar to those of D-loops made from RFI; the complexes were not dissociated by treatment with 0.2% SDS nor by heating at 50 °C for 4 min in 1.5 M NaCl and 0.15 M Na citrate at *p*H 7. Preliminary electron microscopic observations suggest that a significant fraction of complexes made with form II DNA contain D-loops (data not shown). Recently, McEntee, Weinstock and Lehman also described the synthesis of D-loops from non-superhelical DNA, in that case, from linear phage P22 DNA[10].

The formation of D-loops by form II DNA suggests that recA protein unwinds DNA in the presence of ATP as well as in the presence of the analogue, ATPγS.

Discussion

Other experiments have shown that ATPγS competitively inhibits the ATPase activity of recA protein and blocks the formation of D-loops, but promotes the formation of complexes of protein, double-stranded DNA and single-stranded DNA (unpublished observations). The competitive inhibition shows that ATPγS occupies the same binding site as ATP; this, together with the other observations cited, suggests that the accumulation of partially unwound molecules reflects a step in the reaction that normally produces a D-loop. These effects of ATPγS on recA protein are reminiscent of observations on DNA gyrase which also catalyses several partial reactions in the presence of another non-hydrolysable analogue of ATP[11]. From the observed synthesis of D-loops with form II DNA, we infer that recA protein also unwinds DNA in the presence of ATP. The hydrolysis of ATP alters or dissociates the protein–DNA complex and accounts for the failure of partially unwound molecules to accumulate in the presence of ATP and DNA ligase (unpublished observations).

At first glance, the activation of unwinding by heterologous single strands seems incongruous for a mechanism that promotes homologous pairing, but further thought suggests that the opposite is true, that nonspecific stimulation of unwinding by any single strand explains in part how recA protein promotes a search for homology. As recombination in *E. coli* depends strongly on the function of recA[12], the action of recA protein by this mechanism implies that recombination often begins with the interaction of a single strand with duplex DNA.

Mutants of recA are pleiotropic, they affect not only recombination, but also a set of inducible functions, the so-called SOS functions[13]; these include error-prone repair, inactivation of phage repressors and the synthesis of recA protein itself (for reviews see refs 14, 15). Damaged DNA or blocked replication are inducing stimuli, and oligonucleotides have been implicated as intermediates in the induction[16,17]. The *tif*-1 mutation, which is located in the *recA* structural gene, makes the synthesis of recA protein thermo-inducible[18-22], indicating that a suitable form of the protein stimulates its own synthesis. The interaction of oligonucleotides with wild-type protein presumably stimulates synthesis by the same mechanism. However, observations on the *tsl* mutation indicate that abundant synthesis of recA protein may not be sufficient to induce fully all of the SOS functions[19,23]. Do oligonucleotides have a direct role in the inducible functions? Roberts *et al.* have studied one of the SOS functions *in vitro*, namely the inactivation of λ repressor by proteolytic cleavage[24,25]. In spite of the difficulty of reconciling proteolysis and unwinding of DNA, there are marked similarities between our observations on the unwinding of DNA by wild-type recA protein and those of Roberts *et al.* on inactivation of λ repressor by *tif* recA protein. Both processes require ATP or ATPγS, and oligonucleotide or single-stranded polynucleotide. The experiments described in this article show that both oligonucleotides and single-stranded DNA affect the functional properties of recA protein. The stimulation of unwinding by oligonucleotides, as well as by single strands, suggests that a common mechanism relates the function of recA protein in recombination to the other functions that are induced by oligonucleotides. Accordingly, oligonucleotides or single strands may trigger a conformational change of recA protein that can both unwind DNA and alter the interaction of recA protein with certain other proteins that bind to DNA.

A further inference from the similar effect of single strands and oligonucleotides on recA protein is that recombination may be inducible to some extent. Induction of synthesis of recA protein by single strands would provide the requisite amounts of protein to form D-loops, amounts which are large *in vitro*[4]. On the other hand, the induction of recA protein synthesis by oligonucleotides would not necessarily produce an increase in the number of genetic exchanges if any other factor, such as single strands, were limiting.

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The *src* gene product of transformed and morphologically reverted ASV-infected mammalian cells

Marc S. Collett, Joan S. Brugge & Raymond L. Erikson
Department of Pathology, University of Colorado Medical Center, 4200 East Ninth Avenue, Denver, Colorado 80262
Alan F. Lau, Richard A. Krzyzek & Anthony J. Faras*
Department of Microbiology, University of Minnesota Medical School, 925 Mayo Memorial Building, Minneapolis, Minnesota 55455

Morphological revertants of avian sarcoma virus transformed vole cells contain the sarcoma gene product (pp60^{src}) in an enzymatically active form, suggesting that the presence of pp60^{src} protein kinase activity is insufficient to induce morphological transformation. Structural analyses of pp60^{src} from infected vole cell clones suggest that in one of the revertant clones an alteration in pp60^{src} may be responsible for morphological reversion while in a second clone, reversion may result from an alteration in a cell gene product with which pp60^{src} must interact. As these morphological revertant cells are tumorigenic, different cell components are required to interact with pp60^{src} to facilitate the two events.

AVIAN SARCOMA VIRUSES (ASV) are able to induce tumours in birds and a variety of mammals as well as stably transform both avian and mammalian cells in culture. A single ASV gene, designated *src*, has been shown to be responsible for the induction and maintenance of cell transformation *in vitro* and tumour production in infected animals[1,2]. The ASV transforming or *src* gene product is a 60,000-molecular weight (MW) phosphoprotein (pp60^{src})[3-6] with an associated protein kinase activity, suggesting that ASV-induced neoplastic transformation may be the result of aberrant phosphorylation of cellular proteins by pp60^{src} (refs 6–8). In an attempt to elucidate the mechanism(s) by which the expression of this particular viral gene is regulated, and hopefully ultimately provide information concerning virus-induced oncogenesis, we have compared the products of the *src* gene in virus-transformed mammalian cells and morphologically normal revertant subclones of these cells. The cell system chosen for these studies was ASV-transformed

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* To whom reprint requests should be addressed.

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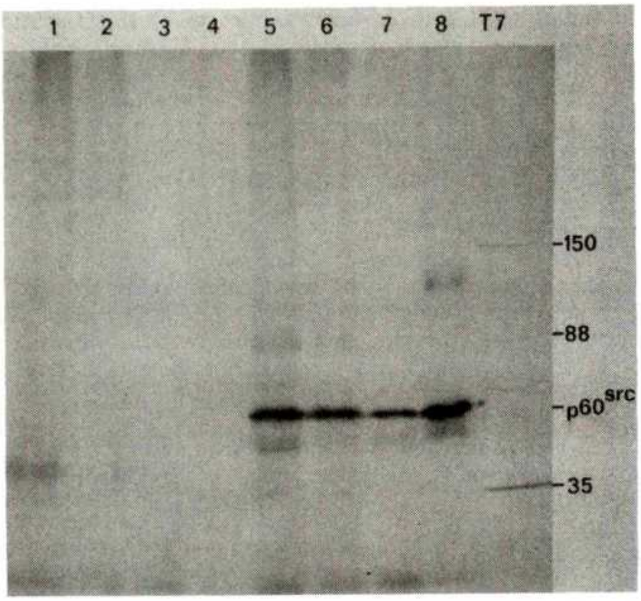

Fig. 1 Presence of phosphorylated pp60^{src} in transformed and revertant vole cells. Cultures were radiolabelled for 2 h with ^{32}P-orthophosphate (carrier-free (ICN), 0.5 mCi ml^{-1}). After cell lysis and clarification, cell extracts were immunoprecipitated with either normal rabbit serum or TBR serum as previously described[3,7]. Portions of the immunoprecipitated proteins were subjected to discontinuous SDS-polyacrylamide gradient gel electrophoresis (5–15% acrylamide)[3], and the fixed, dried gels were autoradiographed. Tracks 1–4, normal rabbit serum; tracks 5–8, TBR serum. Tracks 1, 5, transformed clone 1T; tracks 2, 6, transformed clone 22T; tracks 3, 7, revertant subclone 866R; tracks 4, 8, revertant subclone 4R. ^{35}S-methionine-labelled T7 virion proteins were included as molecular weight markers; numbers in kilodaltons.

European field vole (*Microtus agrestis*) cells, since not only could we obtain revertants of these transformed cells that exhibited the normal cellular morphology, but also these particular cells contained levels of pp60^{src} similar to the transformed vole cells[9–12]. Moreover, as fully infectious transforming ASV can be recovered from the revertant vole subclones, loss of the transformed phenotype did not seem to result from a mutation affecting the *src* gene sequence[9–11]. Thus the reversion phenomenon in this vole cell system appeared to reflect either some post-translational alteration or lack thereof in pp60^{src}, or a defect in a host cell component(s) with which the *src* gene product must interact.

We report here that, although a slight alteration in the primary structure of pp60^{src} obtained from revertant vole cells was detected, this alteration does not seem to be responsible for loss of morphological transformation in the revertant vole cell types, as the slightly altered pp60^{src} molecule was also found in chick cells morphologically transformed by RSV rescued from these revertant vole cell subclones as well as subclones of spontaneous retransformants. Biochemical analysis of pp60^{src} from two revertant vole cell subclones indicates that, in at least one of the clones, morphological reversion is the result of an alteration in a cellular function rather than *src* itself. pp60^{src} isolated from these revertant vole cells exhibits protein kinase activity, suggesting that the presence of such an activity in cells is itself insufficient to induce morphological transformation. More importantly, as both of the revertant vole cells are tumorigenic it seems that one of the phenotypic parameters of cellular transformation can be expressed in the absence of another.

Phosphorylated pp60^{src} in transformed and revertant cells

Once the presence of the *src* gene product in the revertant vole cell clones[12] was established our initial studies were directed at determining whether any subtle differences could be detected in the structure of pp60^{src} obtained from transformed and revertant vole cells. Because the *src* gene product is a phosphoprotein, studies were carried out to determine if differences in phosphorylation existed between pp60^{src} obtained from transformed and revertant vole cells. ASV (Schmidt–Ruppin strain, subgroup D) transformed and morphologically reverted vole cells were radiolabelled with ^{32}P-orthophosphate and cell extracts were prepared by previously described procedures[3,7]. After immunoprecipitation with either non-immune rabbit serum or serum from rabbits bearing ASV-induced tumours (TBR serum)[3], the radiolabelled materials were subjected to electrophoresis in SDS-polyacrylamide gels. TBR serum precipitated a phosphorylated 60,000-MW protein from both transformed and both revertant vole cell lines (Fig. 1), whereas no such protein could be observed when non-immune rabbit serum was used for immunoprecipitation or when uninfected vole cell extracts were immunoprecipitated with this TBR serum. The intensity of the pp60^{src} bands in the autoradiogram suggests that the *src* gene product in both transformed and revertant cells was phosphorylated to a similar extent. Preliminary results have indicated that proteolytic peptides of phosphorylated pp60^{src} from transformed and revertant cell lines are similar. However, more detailed studies concerning the phosphorylation of the *src* protein in this system are now in progress.

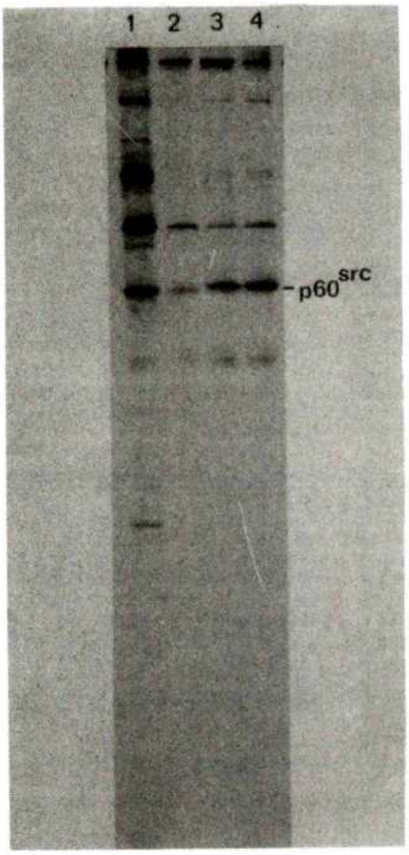

Fig. 2 Detection of a difference in electrophoretic mobility in SDS-containing polyacrylamide gels of pp60^{src} from transformed and revertant vole cells. ^{35}S-methionine labelled samples immunoprecipitated with TBR serum as described in Fig. 1 were electrophoresed in a discontinuous SDS-containing polyacrylamide slab gel consisting of a 4.2% acrylamide, 0.1% bisacrylamide stacking gel and a 10% polyacrylamide (38:1 acrylamide: bisacrylamide) separation gel, 14 cm long. Track 1, transformed clone 1T; track 2, transformed clone 22T; track 3, revertant subclone 866R; track 4, revertant subclone 4R.

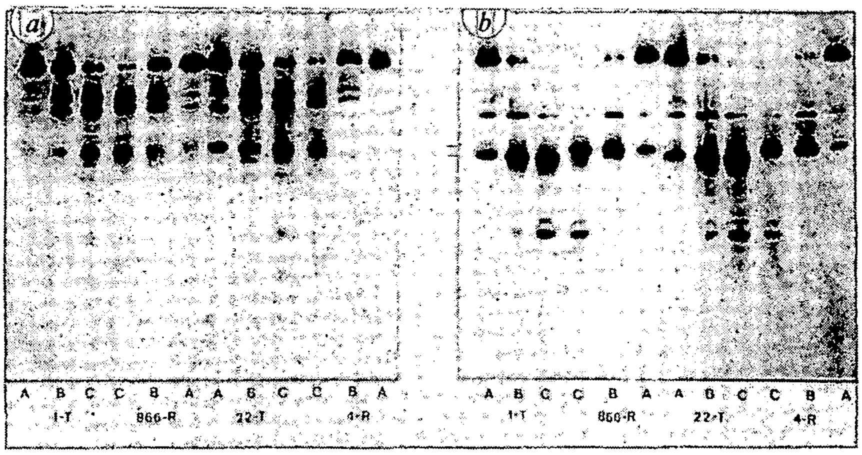

Fig. 3 Partial proteolysis mapping of pp60^{src} from transformed and revertant vole cells. ^{35}S-methionine labelled pp60^{src} proteins immunoprecipitated from the various vole cell lines were excised from preparative polyacrylamide gels and subjected to limited proteolysis during re-electrophoresis as described previously[13]. *a*, Digestion with chymotrypsin. Tracks A, 0.1 μg enzyme; tracks B, 0.6 μg enzyme; tracks C, 2.5 μg enzyme. *b*, Digestion with *Staphylococcus aureus* V8 protease. Tracks A, 0.001 μg enzyme; tracks B, 0.005 μg enzyme; tracks C, 0.05 μg enzyme. The lines in the margin of *b* indicate the V8 protease 26,000-MW peptide (the C-terminal 40% of the pp60^{src} protein molecule)[14] which shows an altered mobility between the revertant and transformed cell proteins.

Difference in electrophoretic mobility of pp60^{src} from transformed and revertant cells

^{35}S-methionine labelled proteins were also immunoprecipitated from extracts of both clones of transformed and revertant vole cells and electrophoresed on a slab gel longer than used in our initial studies in an effort to better resolve the pp60^{src} proteins (Fig. 2). The pp60^{src} proteins isolated from the two transformed cell lines co-migrated, as did the proteins from the two revertant subclones. However, the proteins from the revertant cell lines showed a slightly slower electrophoretic mobility than those from the transformed clones. As the pp60^{src} proteins from transformed vole and chicken cells co-migrate in these conditions (data not shown), these results suggest that the pp60^{src} protein present in the two revertant clones may represent an altered *src* gene product.

Proteolysis mapping of pp60^{src}

For a better assessment of the similarities and any possible differences in the pp60^{src} proteins from the transformed and revertant vole cells, one-dimensional peptide analyses were performed on ^{35}S-labelled pp60^{src} with both chymotrypsin (Fig. 3*a*) and *Staphylococcus aureus* V8 protease (Fig. 3*b*)[13]. The chymotrypic peptides generated by this partial proteolysis of the four proteins were all very similar if not identical (Fig. 3*a*). The V8 protease digestion patterns of pp60^{src} from the two transformed cell lines also appeared identical (Fig. 3*b*). However, both revertant cell pp60^{src} proteins exhibited V8 protease peptides that differed in electrophoretic mobility from the corresponding peptides of the two transformed vole cell lines (Fig. 3*b*, margin lines). The initial digestion products resulting from V8 protease digestion of pp60^{src} are two peptides of MW ~34,000 and 26,000 (ref. 14). The larger V8 protease fragment represents the amino-terminal 60% of the pp60^{src} molecule and the smaller fragment encompasses the carboxy-terminal 40% (ref. 14). Thus, it seems that the pp60^{src} C-terminal V8 protease peptide in both of the revertant cell lines was changed to a peptide of slightly slower electrophoretic mobility, confirming the slight alteration in pp60^{src} obtained from these revertant cells observed by polyacrylamide gel electrophoresis (Fig. 2).

In an attempt to determine if the altered V8 protease peptide of pp60^{src} from the two revertant subclones represented either a stable genetic change, or a variation in secondary protein modification or processing by the revertant cells, virus was rescued from the two revertant subclones and used to infect chicken cells. The ^{35}S-methionine-labelled pp60^{src} proteins were then isolated from these rescued virus-infected (and transformed) avian cells and subjected to partial proteolysis mapping using V8 protease as described above. When the protein obtained from the revertant subclone 4R rescued virus-infected chick cells (4R chick) was analysed, peptide fragments identical to those of pp60^{src} from the 4R revertant vole cells (4R vole) were obtained (Fig. 4*a*), suggesting that the alteration in the C-terminal V8 protease peptide of the *src* protein in this revertant subclone represented a stable change. In addition, we have been able to isolate clones of cells from cultures of our revertant cells grown to high cell density that appear to have regained the transformed phenotype. These re-transformed (RT) clones have also been used for *src* protein analysis. V8 protease peptide mapping of pp60^{src} from a re-transformed clone of the 4R revertant cells (4RT vole) again revealed no change from the pattern observed in the protein from the parent revertant cell line (Fig. 4*a*).

Similar peptide analyses were performed on the *src* protein isolated from chick cells transformed with revertant subclone 866R rescued virus (866R chick) and a clone of re-transformed

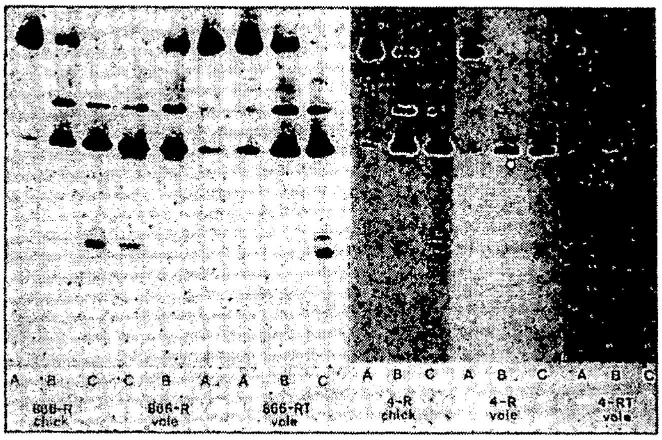

Fig. 4 Partial proteolysis mapping of pp60^{src} from rescued virus-infected chick cells and re-transformed vole cells. The rescue of ASV from the revertant vole cell subclones 4R and 866R by Sendai virus-mediated fusion with chick fibroblasts was performed by the procedure of Boettiger[19] as previously described[9]. The rescued virus (SR-ASV, subgroup D) obtained from the supernatant fluids of the fusion culture was used to infect chick cells. The infection resulted in virus production and the complete transformation of the chick cell culture. Re-transformed cell lines (4RT and 866RT) were obtained by picking cells that appeared morphologically transformed from 4R and 866R revertant subclones that were grown and maintained at high cell densities. The re-transformed cell lines maintain their transformed morphology on continuous subculturing. ^{35}S-methionine labelled pp60^{src} was immunoprecipitated from the rescued virus-infected chick cells (4R chick and 866R chick), the re-transformed vole cells (4RT and 866RT vole), and the original revertant subclones (4R vole and 866R vole) and subjected to V8 protease partial proteolysis as described in Fig. 3 legend. Tracks A, 0.001 μg enzyme; tracks B, 0.005 μg enzyme; tracks C, 0.05 μg enzyme.

vole cells from this revertant cell line (866RT vole) (Fig. 4*b*). As was the case with the 4R revertant cells the two major V8 protease peptides of pp60^{src} from these cell types were all identical. Although it first appeared that this revertant cell clone might be similar to the 4R clone, a closer inspection of the peptide maps of the 866R clone revealed that a slight, but reproducible, difference did exist between the two revertant vole cell subclones. This difference reflected the presence of an additional peptide band immediately below the major C-terminal V8 protease fragment in the parental 866R revertant vole cell line (Figs 3 and 4*b*). Moreover, this particular peptide was absent in V8 protease hydrolysates of pp60^{src} from either chick cells infected with RSV rescued from the 866R cells or spontaneous re-transformants of this revertant vole cell clone (Fig. 4*b*). Although it may be involved in the morphological reversion of this cell line, the significance of this extra peptide is unclear. The presence of the peptide only in the revertant vole cell pp60^{src} and not in the *src* proteins derived from the rescued virus-infected (and transformed) chick cells and the re-transformed vole cells suggests that this alternation is not genetically stable and may be specific for the pp60^{src} of this revertant line. Nevertheless further studies are needed to determine whether this peptide represents expression of a *src* gene from a second ASV present in this particular revertant cell line.

Table 1 Quantitation of pp60^{src} protein and pp60^{src}-associated protein kinase activity in transformed and revertant vole cells

Cell line	pp60^{src} Protein (μg per mg cell protein)	Specific activity of pp60^{src} protein kinase (fmol ^{32}P incorporated per μg pp60^{src})	Total pp60^{src} protein kinase activity (fmol ^{32}P incorporated per mg cell protein)
Transformed 1T	0.30	135	40.5
Transformed 22T	0.07	383	26.8
Revertant 866R	0.21	321	64.2
Revertant 4R	0.13	259	33.7

Cultures were labelled with 25 μCi ml^{-1} of ^{35}S-methionine for 2 h, and cell extracts were prepared as described previously[3,7]. Samples were taken for the determination of protein content, and portions were immunoprecipitated with TBR serum. The amount of pp60^{src} protein in each extract was determined as previously described[18]. The resulting kinase activities, determined by quantitation of the phosphorylated IgG bands from polyacrylamide gels, were normalised with respect to the amount of cell extract protein used for the immunoprecipitation.

pp60^{src}-associated protein kinase activity

As the pp60^{src} proteins from both revertant cell lines appeared structurally different from those obtained from the two transformed vole cell lines, we attempted to determine if they were functionally altered. It has been found[6-8] that pp60^{src} is associated with a protein kinase activity. Using what is now considered to be the conventional assay for pp60^{src} protein kinase activity, we have found that both revertant, as well as both transformed, vole cell lines show pp60^{src}-protein kinase activity (Table 1). No correlation can be made between the concentration of the *src* protein or the specific activity of pp60^{src}-protein kinase and the transformed or morphologically reverted state of the ASV-infected vole cells (Table 1).

Discussion

The slower electrophoretic mobility of the C-terminal V8 protease peptide of pp60^{src} from the revertant subclones represents a stable genetic change; however, it does not seem to impair the ability of the protein to induce morphological transformation. It is possible that the revertant subclones studied here were originally infected with a viral variant of SR-ASV or a different, contaminating strain of ASV, and that this is the reason for the differences in the pp60^{src} peptide maps. This seems unlikely as the immunoprecipitation with our TBR serum is strain specific for pp60^{src} of SR-ASV[16,17], and neutralisation data indicate that the rescued viruses obtained from the two revertant cell lines are the SR subgroup D strain of ASV. Peptide analyses on the *src* protein of other strains of ASV (Prague, Bratislava, Bryan) have shown that some variation in the V8 protease peptide maps does exist[17]. However, none of the viral pp60^{src} proteins studied so far have exhibited the peptide pattern (altered mobility of the C-terminal V8 protease peptide) of the proteins obtained from the two revertant vole cell lines studied here. It is of interest that a normal avian cell protein related to pp60^{src}, designated pp60^{sarc}, does have a C-terminal V8 protease peptide of the same electrophoretic mobility as those of the *src* proteins of the two revertant cell lines[18]. Additional subclones of revertants are now being isolated and peptide analyses of their pp60^{src} proteins being performed to determine if morphological reversion is always accompanied by the change in the C-terminal V8 protease peptide.

Although the pp60^{src} proteins in the transformed and revertant vole cells appear structurally different, they all exhibit protein kinase activity. It is possible that the *src* function involved in the changes in cellular morphology is not the pp60^{src}-associated protein kinase. It is also highly possible that pp60^{src} has other enzymatic activities. Alternatively the change in the *src* protein of the revertant cells may have altered *src* kinase specificity and/or cellular target recognition. Finally, the change in the pp60^{src} protein of the revertant cells may have nothing to do with the reversion process, but rather changes in the cells themselves have resulted in the ineffective action by the *src* protein in morphological transformation. For example, a cellular substrate of pp60^{src} may have been altered in such a way that it is no longer phosphorylated by the *src* kinase. This possibility appears highly plausible as the pp60^{src} protein from both revertant vole cell clones are able to cause morphological transformation in chicken cells and in retransformed vole cells. Thus, the transformed and revertant vole cells should provide an appropriate system to facilitate the identification of the target(s) of the ASV *src* gene product.

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letters

Is Z Cha another case of gravitational radiation?

H. Ritter

Max-Planck-Institut für Astrophysik, Föhringer Ring 6, D-8000 München 40, FRG

Z Cha is an eclipsing cataclysmic binary with an extremely short orbital period of only $P = 0.07449923$ d (ref. 1). Cataclysmic binaries are systems consisting of a white dwarf primary, of mass M_1, and of a secondary of low mass M_2, which fills its critical Roche volume[2]. The secondary feeds an accretion disk around the primary by transferring matter through the inner lagrangian point L_1. At the point where the streaming matter impacts the disk, a shock front is formed which is usually referred to as the hot spot. The geometry of the system, as seen from above the orbital plane, is shown in Fig. 1. The determination of the masses, the absolute dimensions and the mass exchange rate of Z Cha are reported here. Various possible mechanisms for the mass exchange are discussed which lead to the conclusion that it is probably due to the action of gravitational waves.

High-speed photometry observations[1] of Z Cha have clearly shown that two separate emitting regions undergo phase shifted total eclipses. These emitting regions are the hot spot and the centre of the accretion disk including the white dwarf primary. The half widths[4] of the white dwarf eclipse, Δt_{WD}, and of the hot spot eclipse, Δt_{HS}—as well as their mutual phase shift δt—have been determined from improved photometric observations[3]. In addition the durations of the partial phases of the corresponding eclipses, Δ_{WD} and Δ_{HS} have been estimated[5]. The resulting numerical values of Δt_{WD}, Δt_{HS}, δt, Δ_{WD} and Δ_{HS} are listed in Table 1*a*. Recent spectroscopic observations[6] have enabled the radial velocity K_1 and the projected orbital velocity of the accretion disk $v \cdot \sin i$ to be determined. These quantities are also listed in Table 1*a*.

The masses and the absolute dimensions of the system can be determined directly if the standard assumption[7] is made, namely that $v \cdot \sin i$ is the keplerian rotation velocity at the hot spot's distance, d, from the centre of the disk. Analysis of the double eclipse using a method described elsewhere[4] yields two equations for the mass ratio M_1/M_2 and the orbital inclination i in terms of R_2/A and R_2/A_S. Using geometrical relations shown in Fig. 1 and those of the keplerian orbit these equations can be solved[5]. The resulting main parameters of Z Cha are listed in Table 1*b*. The radii of the white dwarf primary, R_1, and of the hot spot, R_{HS}, have been computed from the durations of the partial phases of the corresponding eclipses[5].

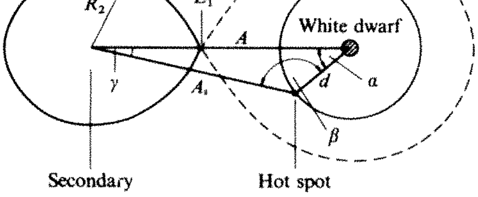

Fig. 1 Geometry of Z Cha as seen from above the orbital plane. Except for the radius of the hot spot the drawing is to scale.

Most remarkably, although the secondary was not assumed to be a main sequence star (an assumption usually made when discussing cataclysmic binaries[2,7]) it turns out that the secondary lies almost exactly on the theoretical mass–radius relation of low mass main sequence stars[8]. Even more surprisingly the primary fits the theoretical mass–radius relation of white dwarfs[9,10]. Both these findings are not self-evident and must be considered as a confirmation of the reliability of the parameters given in Table 1. An additional consistency check is provided by comparing the value of α (see Fig. 1) given in Table 1*b* with the value $\alpha \simeq 34°$ derived from observations[1]. The agreement is quite good. (Note that there apparently is an error in ref. 1 and that in fact $\alpha = 34°$ and not $\alpha + \gamma = 34°$; details will be given elsewhere[5].) Moreover, if Δt_{HS} is replaced by α (34°) as an input parameter in the above analysis the values of all the resulting parameters are almost identical to those given in Table 1*b*.

It is now possible to estimate the rate of mass exchange, $\dot{M}$, by determining the luminosities of the hot spot and/or of the accretion disk. The rather lengthy procedure necessary to transform relative amplitudes of the observed light curves[1] into absolute luminosities is described elsewhere[5]. Basically the procedure involves four steps. First, the relative amplitudes of the different emitting regions, that is, of the secondary, the hot

Table 1 Parameters for Z Cha

a Observational data for Z Cha*

Quantity	Symbol	Value	Ref.
Orbital period	P	0.07449923 d	1
	Δt_{WD}	(350 ± 10) s	3†
	Δt_{HS}	(585 ± 10) s	3†
	δt	(226 ± 10) s	3†
Defined in text	Δ_{WD}	(110 ± 10) s	3†
	Δ_{HS}	(190 ± 10) s	3†
	K_1	(122 ± 6) km s^{-1}	6
	$2 \cdot v \cdot \sin i$	(1,217 ± 54) km s^{-1}	6

b Resulting physical parameters for Z Cha

Orbital inclination	i	74° ± 0.8°	This paper
Mass ratio	M_1/M_2	2.2 ± 0.3	
Mass of the primary	M_1	$(0.35 \pm 0.06) M_\odot$	
Mass of the secondary	M_2	$(0.16 \pm 0.02) M_\odot$	
Radius of the primary	R_1	$(0.018 \pm 0.002) R_\odot$	
Radius of the secondary	R_2	$(0.19 \pm 0.01) R_\odot$	
Radius of the hot spot	R_{HS}	$(0.032 \pm 0.003) R_\odot$	
Orbital separation	A	$(0.59 \pm 0.03) R_\odot$	
Radius of the disk	d	$(0.16 \pm 0.01) R_\odot$	
	α‡	39.8° ± 2.7°	
	β‡	127.5° ± 3.0°	

* Errors indicated are estimated 1σ (except for K_1 and $v \cdot \sin i$ (ref. 6)).

† Determined from the light curve given in ref. 3.

‡ Defined in Fig. 1.

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spot and the accretion disk including the white drawf primary, have been determined from observations of Z Cha at minimum light[1]. Second, the measured amplitudes of the different emitting regions in 'white light' (ref. 1) have been converted to relative bolometric amplitudes, taking into account the different effective temperatures of the different emitting regions. Third, an absolute luminosity for one of the emitting regions must be determined. This has been done by assuming that the secondary is a normal main sequence star[8] as indicated by its position in the mass–radius diagram. Finally the absolute luminosities of the hot spot and of the accretion disk have been computed and directly converted into accretion rates[5,11]. The accretion rates determined in this way, $\dot{M}_{\mathrm{HS}}$ from the hot spot luminosity and $\dot{M}_{\mathrm{D}}$ from the disk luminosity, are listed in Table 2. The errors given are purely formal, the rates themselves are probably known only within a factor of 2.

Knowing the approximate accretion rate, what mechanism can give rise to a corresponding mass exchange rate? The most obvious mechanism, namely nuclear evolution of the secondary, can be ruled out because the secondary has a mass of only $\sim 0.16 M_{\odot}$, and is almost certainly fully convective[8] and thus remains chemically homogeneous throughout its evolution. This has the effect of decreasing the star's radius with time. Thus no mass exchange can be expected in these circumstances. However, even if the secondary were not fully convective it would increase its radius a nuclear time scale $\tau_{\mathrm{nuclear}} \approx 10^{12}$ yr giving rise to a mass exchange rate of only $\dot{M} \approx 2 \times 10^{-13} M_{\odot}\,\mathrm{yr}^{-1}$. This is two orders of magnitude less than that estimated from observations.

The second likely mechanism, namely mass loss by the secondary on a thermal time scale, due to thermal readjustment, can also be ruled out. This is because the system, which has a mass ratio $M_1/M_2 \approx 2.2$, is secularly stable against mass exchange unless the mass–radius exponent of the secondary $\alpha_2 = d \log R_2 / d \log M_2$ is less than -0.7. However, the corresponding value of α_2 for a $0.16 M_{\odot}$ main sequence star is $\alpha_2 \approx +0.7$. In fact, even in the worst case of deviation from thermal equilibrium, that is when the fully convective star expands adiabatically, α_2 is only $-1/3$ and the system would still be secularly stable against mass exchange.

Thus the most obvious, classical mechanisms of mass exchange are insufficient to explain the observations of Z Cha. On the other hand, it has been suggested that cataclysmic variables, such as Z Cha, should exchange matter as a consequence of angular momentum loss due to gravitational radiation[12]. (Note that this mechanism has the *a priori* advantage of always forcing the binary to some mass exchange independent of the mass–radius exponent of the mass-losing star.) According to Einstein's quadrupole formula[13], gravitational waves should give rise to a mass exchange rate[12]

$$\dot{M}_{\mathrm{GR}} = \frac{32 G^3}{5 C^5} (M_1 + M_2) M_1 M_2^2 A^{-4} \times \left[\frac{M_1 - M_2}{M_1} + \frac{1}{2} \left(\alpha_2 + \frac{M_1 + M_2}{M_1} \frac{d \log f_2}{d \log (M_1/M_2)} \right) \right]^{-1} \qquad (1)$$

where f_2 is the Roche radius of the secondary in units of the system's separation A. The numerical value of $\dot{M}_{\mathrm{GR}}$ corresponding to the parameters listed in Table 1*b* is given in Table 2 and can thus be compared directly with the estimates $\dot{M}_{\mathrm{D}}$ and $\dot{M}_{\mathrm{HS}}$. Gravitational radiation apparently induces just that amount of mass exchange—within the uncertainties—estimated from observations. Moreover, unless one were prepared to accept rather exotic mechanisms for the mass exchange the only mechanism capable of explaining mass exchange at the observed level in a natural way is loss of angular momentum due to gravitational radiation. It can therefore be concluded that Z Cha is, in addition to the binary pulsar[14], another example of the action of gravitational radiation.

Table 2 Mass exchange rates

Quantity	Value ($\times 10^{-11} M_{\odot}\,\mathrm{yr}^{-1}$)
$\dot{M}_{\mathrm{D}}$*	1.9 ± 1.3
$\dot{M}_{\mathrm{HS}}$†	4.3 ± 1.2
$\dot{M}_{\mathrm{GR}}$	4.2 ± 1.6

* Estimated from luminosity of the accretion disk.
† Estimated from luminosity of the hot spot.
‡ Expected from the action of gravitational radiation.

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Dissipation, merging and the rotation of galaxies

S. Michael Fall

Institute of Astronomy, University of Cambridge, Madingley Road, Cambridge and Center for Astrophysics, Cambridge, Massachusetts

The distinction between elliptical and disk galaxies is often explained by invoking different rates of dissipation and star formation in the proto-galactic gas clouds from which they are presumed to have formed[1–3]. Another possibility is raised by the suggestion that many ellipticals are the remnants of galaxies that merged while suffering the tidal distortion and violent relaxation of a slow encounter[4,5]. Various *N*-body simulations indicate that merging can account for many of the observed properties of bright elliptical galaxies[6–9], although some reservations have been expressed about the possibility of accounting for their low rotation velocities[10]. My aim here is to consider a specific model for the formation of disk galaxies by dissipation and the formation of bright ellipticals by merging and to suggest that it can account for their rotation properties in a natural way.

The rotation of a self-gravitating body is conveniently expressed in terms of the dimensionless spin parameter $\lambda \equiv J |E|^{1/2} G^{-1} M^{-5/2}$ where J, E and M are respectively the total angular momentum, energy and mass of the body and G is the gravitational constant. In the hierarchical clustering picture, all structures are endowed with some rotation before collapse as a consequence of their mutual tidal torques. The characteristic spin of this mechanism is $\lambda_{\mathrm{T}} \simeq 0.07$ and any significant differences from this value must be the result of non-gravitational effects[11,12]. An elliptical galaxy has $\lambda \simeq 0.3 (v_r / \sigma_r)$ where v is the peak rotation velocity along the major axis and σ_r is the line-of-sight velocity dispersion near the centre of the galaxy[6]. The measurements of these velocities for 25 bright ellipticals[13–17] give the average spin value $\lambda_{\mathrm{E}} \simeq 0.08$ and the distribution shown in Fig. 2 (neglecting small corrections for the

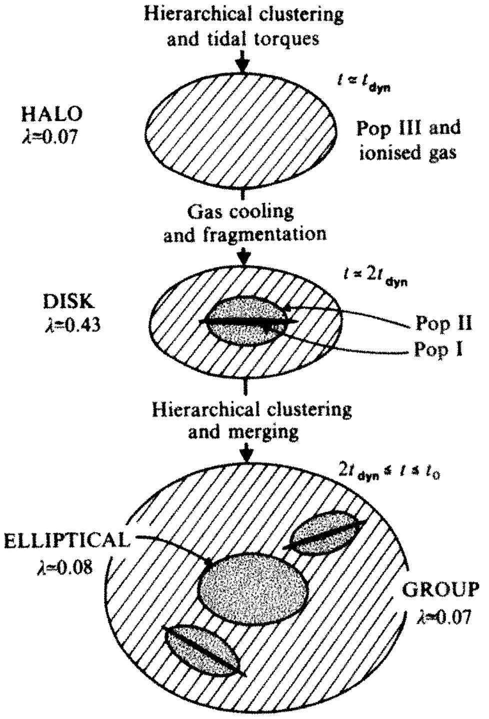

Fig. 1 Evolutionary sequence discussed in the text. Pre-galactic haloes are formed by the hierarchical clustering of dark stars and gas; disk galaxies result from dissipation and fragmentation within the haloes; elliptical galaxies are produced by merging in small groups.

orientations of the galaxies and uncertainties in the observations). Disk galaxies appear to be supported by rotation alone and, to the extent that this is true, have $\lambda_{\rm D} \simeq 0.43$. (This follows from equations 9, 11 and 14 of ref. 18, equation 6 on page 386 of ref. 19 and the virial theorem; it is exact for a cold exponential disk without a bulge component.)

The proposed evolutionary sequence, illustrated in Fig. 1, is based on the picture of galaxy formation and clustering outlined by White and Rees[20]. In their view, pre-galactic haloes formed by gravitational clustering and consisted of low-mass or burnt-out stars (pop III) along with some residual gas. If the gas was originally ionised (for example by shocks), the condition that it could cool and collapse within the dark haloes sets their characteristic mass and (half-mass) radius at $M_{\rm H} \simeq 5\times 10^{12} M_\odot$ and $R_{\rm H} \simeq 150$ kpc (to rough accuracy[20]). The most likely outcome of this dissipative process would seem to be a population of disk galaxies[21] (spirals if some gas was retained, lenticulars if it was depleted or removed). Their characteristic masses and radii must have been set by the poorly understood details of the collapse process and will therefore be parametrised in terms of the halo values: $M_{\rm D} \simeq xM_{\rm H}$ and $R_{\rm D} \simeq yR_{\rm H}$. On observational grounds[18,22–24], the values $x \simeq y \simeq 0.1$ are indicated and they satisfy the condition that the gas was able to reach a self-gravitating state before fragmenting into stars. The tidal spin of the haloes and the condition that angular momentum was conserved during the collapse of the gas now lead to the characteristic spin $\lambda_{\rm D} \simeq (xy)^{-1/2}\lambda_{\rm T}$ for disk galaxies (neglecting geometrical factors of order unity). This estimate is based on the suggestion that any mechanisms for the transport of angular momentum (such as turbulent viscosity) would not have been efficient during collapse[25], and it agrees with the empirical spin value for $xy \simeq 0.03$.

Gravitational clustering on scales larger than the individual haloes would not have been influenced by their internal dissipation and would have given rise to the observed hierarchy of groups and clusters[26,27]. The characteristic masses and radii of the groups are related to the number of member galaxies m by the expressions $M_{\rm G} \simeq mM_{\rm H}$, $R_{\rm G} \simeq m^{\alpha+1/3}R_{\rm H}$ where $\alpha \simeq 0.3$–0.5 is the index of the 'initial' perturbation spectrum $\delta\rho/\rho \propto M^{-\alpha}$. The groups of the first generation to reach equilibrium would have had about $m \simeq 2$–8 members and tidal stripping is expected to have detached most of the haloes from their galaxies in a fraction $0.08R_{\rm G}/R_{\rm H} \simeq 0.08m^{\alpha+1/3}$ of the first crossing time (equation A4 of ref. 20). This is expected to have left each group with a fairly smooth background of stripped halo material and to have left most of the luminous parts of the galaxies relatively undisturbed (so long as they are tightly bound). In this environment, the merging time scale for the galaxies themselves is about $0.03x^{-1}(M_{\rm G}/M_{\rm D}) \simeq 0.03mx^{-2}$ group crossing time scales (equation A7 of ref. 20) so about one merger per group is expected before its incorporation into the next level of the hierarchy. After that, the conditions for merging are less propitious, especially in large clusters, because merging cannot occur if the encounter velocities of the interacting galaxies are more than a few times their internal velocity disperions[6,7] ($\sim$250 km s^{-1}). This condition also implies that the ellipticals produced from n disk galaxies have characteristic masses and radii with the approximate values $M_{\rm E} \simeq nM_{\rm D}$ and $R_{\rm E} \simeq nR_{\rm D}$.

The spins of elliptical remnants can include contributions from both the orbital and the internal angular momenta of the disk galaxies that produced them. To the extent that the merging process was hierarchical, the internal contribution is negligible because the galaxies most likely to merge were those that formed near each other and therefore provided the mutual torques responsible for their equal and opposite tidal spins. Some of the merging in large groups may, however, have involved galaxies that did not form near enough to guarantee this cancellation. If they had no correlation, the typical contribution was $n^{1/2}J_{\rm D}$ to the angular momentum of a remnant and therefore $n^{-3/2}\lambda_{\rm D}$ to its spin. Thus, even in this extreme case, the internal contribution becomes negligible after about two merger

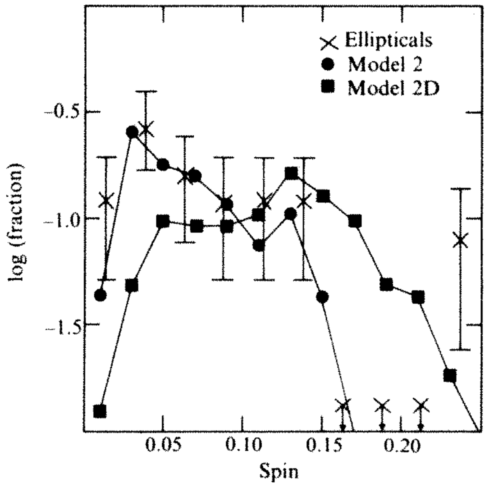

Fig. 2 Spin distributions. The normalised histogram for elliptical galaxies is from the data of refs 13–17 (listed also in Table 2 of ref. 9). The other histograms are for merger remnants at the end of two 1,000-body simulations that differ only in the initial spin values of their particles: $\lambda = 0.08$ for model 2 (one of the 'standard' simulations of ref. 9) and $\lambda = 0.40$ for model 2D (a new simulation with the same particle sizes, masses and orbits). The initial spin orientations are random and the final spin distributions are similar because of the statistical $n^{-3/2}$ dependence of the contribution from internal spins. Orienting the initial spins to cancel in pairs (by earlier tidal torques) would result in even more similar final distributions. The spin distributions are not sensitive to the sizes of the particles but they do depend somewhat on the initial position and velocity distributions (Poisson positions and Hubble velocities here). These simulations do not include a component of stripped halo material; however, the spins of merger remnants are not expected to depend sensitively on the presence of this component so long as it also participates in the hierarchical development of clustering.

events ($n \simeq 3$). The orbital contribution to the spins of elliptical remnants must have derived from tidal torques in the clustering hierarchy, with a maximum value set by the condition that interacting galaxies should have overlapped enough to have merged within a few orbital periods. (Otherwise, the orbits could have been disrupted and merging prevented.) The simulations of merging pairs of galaxies[6,7] indicate $\lambda_{\max} \simeq 0.2$ (for parabolic encounters with zero net internal spin) and the cosmological simulations[8,9] indicate that the average spin value of merger remnants is about $\frac{1}{2}\lambda_{\max} \simeq 0.1$. This is because the distribution of impact parameters is fairly flat and most merging is from marginally bound orbits. Figure 2 shows the results of two simulations by Dr S. J. Aarseth and myself; the agreement with the spin distribution of real ellipticals is reasonable in both cases.

In many respects, the specific model outlined here is undoubtedly over-simplified and leaves some important questions unanswered: How did the dwarf ellipticals form? Why are ellipticals virtually gas-free? What determines the colours of elliptical remnants? The model suggests that different dissipation and star formation rates need not be invoked at the formation epoch of bright galaxies to explain their later morphological differences. The real situation may, however, have involved a variety of galaxy formation processes with merging only responsible for the making of some ellipticals. Perhaps the main conclusion to be drawn from the line of reasoning presented here is that the low rotation velocities of bright elliptical galaxies are no embarrassment to the idea that they were produced by the merging of rapidly rotating disk galaxies.

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The vertical structure and thickness of Saturn's rings

Jeffrey N. Cuzzi*, Joseph A. Burns†, Richard H. Durisen‡ & Patrick M. Hamill§

* Ames Research Center, NASA, Moffett Field, California 94035
† Cornell University, Ithaca, New York 14850
‡ Indiana University, Bloomington, Indiana 47401
§ Langley Research Center, NASA, Hampton, Virginia

Observations of Saturn's rings over the last few years have contributed greatly to unravelling questions as to their nature and origin. However, some fundamental issues concerning the vertical structure and thickness of the rings have remained in dispute. In this note we present a synthesis of our results which reconciles most of the conflicting data. We first discuss the vertical structure and show how the many-particle-thick model appears to be feasible, but permits a local ring thickness of only a few tens of metres, much less than the thickness suggested by observations at ring-plane passage. We then describe how solar and satellite perturbations, which are coherent and not dispersive, do not significantly affect the true local vertical thickness of the rings, but do affect the tilt of the mean ring plane in a way that could be relevant to an understanding of the ring-passage observations.

One outstanding problem is the finding that the physical thickness of the saturnian rings is far too small to be directly observed. From measurements of the minimum brightness of the edge-on rings during ring-plane passage in 1966, ring thicknesses of 2.8 ± 1.5 km (ref. 1) and 1.4 ± 0.5 km (ref. 2) have been inferred. Reanalysis of these data[3], however, gives a thickness of 0.8 (+2.3, −0.8) km, implying that only an upper limit on the thickness of ~3 km may be confidently claimed.

A further question concerns the local vertical structure of the rings—are they many particles thick or are they essentially a monolayer? A ring that is many particles thick is the classical solution to the observed opposition effect[4]. Such a thickened ring implies random velocities with respect to the average local motion, and yet such velocities are continually damped by inelastic collisions between particles[5]. Such collisions occur about once per orbit for an optical depth of the order of unity. As a result, an originally 'thick' ring system flattens—in a time that is extremely short compared to 4.5×10^9 yr—to a layer that is at most a few particles thick unless the random motions are sustained by some ongoing source of energy. Obviously, in an essentially flat system, the ring thickness is of the order of the particle size. Thus, the above dynamical arguments and ring thickness 'observations' imply a monolayer with thickness (particle size) ~1 km; such a thickness is difficult to reconcile with either the opposition effect or with recent microwave observations[6,7].

Mechanisms considered as possible sources of energy for continual thickening of the rings include collisions among the particles[4,8–10] and perturbations of the system by the saturnian satellites and the Sun[4,10,11]. In recent studies[12,13] we have discussed these and less significant mechanisms, such as meteoroid bombardment and radiation pressure, in detail.

Although, as argued above, collisions quickly flatten a ring system, the flattening proceeds, at most, only to the point where the system is a few particles thick. This is easily understood: imagine a ring system, nearly a monolayer, of identical particles of radius R in an orbit with semimajor axis a about a planet. Because of their keplerian motion, neighbouring particles will have a relative tangential velocity $\sim \omega R$, where ω = local orbital angular velocity. Particles collide because of this shear motion and their finite radii and soon develop an out-of-plane velocity component comparable to the shear velocity difference ωR over a particle diameter. By conservation of angular momentum in such collisions, the rings spread radially, in the absence of other restrictions, until the particles no longer collide. Such an evolution—rapid collisional flattening to a thickness of a few particle radii, followed by a very gradual spreading—was introduced by

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Jeffreys[5], treated numerically by Brahic[9], and theoretically by Cook and Franklin[7] and Goldreich and Tremaine [10] with similar results. We also have investigated thickening mechanisms based on this 'Kepler shear' but applied to a ring system composed of particles of size distribution n_0R^{-3}, which is that expected for an ensemble of particles which, perhaps only very early in their history, but possibly also at present, undergo comminution and mutual fragmentation. A size distribution of this general type seems to be required to explain the wavelength independence of the radar reflectivity of the rings[6,7]. We have shown[12] that the ring system attains a steady state in which gravitational scattering by the large particles imparts random velocity components V_R to the small particles while inelastic particle collisions decrease these random velocities. This leads to a gaussian distribution $\exp(-z^2/z_0^2)$ of particle density with vertical displacement $z \sim aV_R/\omega a$.

Our results are not sensitive to the form of the size distribution. Suppose $n(R) = n_0R^{-p}$ with minimum and maximum particle radii $R_{\min}$ and $R_{\max}$. The energy balance equation for the random particle motions is of the general form[12]

$$f_1 = \frac{\int M^2 n(M)\,\mathrm{d}M}{\langle V_R \rangle^3}$$

where the constant f_1 includes the effects of inelastic collisions and Kepler shear, and depends only on ω, normal optical depth τ_0, and coefficient of restitution e. We may obtain the particle mass distribution $n(M)$ from $n(R)$, and we obtain n_0 from τ_0. Then,

$$n(M) = f_2 n_0 M^{-(p+2)/3}$$

where

$$n_0 = \frac{f_3(2-p)}{z_0(R_{\max}^{(3-p)} - R_{\min}^{(3-p)})}$$

and f_2, f_3 are constants.

Integrating over the size distribution, and using $z_0 = \sqrt{2}\langle V_R \rangle/\omega$, we obtain

$$z_0 = KR_{\max},\ p \leqslant 3$$

$$z_0 = K\left(\frac{R_{\max}}{R_{\min}}\right)^{(3-p)/4} R_{\max},\ 7 \geqslant p > 3$$

$$z_0 = KR_{\min},\ p > 7$$

where K varies only slowly with p, τ_0 and e. The result is independent of the exponent p of the size distribution for $p \leqslant 3$. If $(R_{\max}/R_{\min}) \leqslant 10^2$ or 10^3 (ref. 6), z_0 is still comparable to $R_{\max}$ for $p \leqslant 4.5$. Evaluating the constant K for $p = 3$ (ref. 12), we find that $z_0 \sim 5\ R_{\max}$.

Thus, although the full ring thickness is only about 10 times the radius of the largest particles, the ring is many small particles thick. Because the small particles dominate the surface area for an R^{-3} distribution, this can account for the optical observations[4,14] and microwave scattering behaviour[6,7]. An estimate of the maximum size of the ring particles following the R^{-3} size distribution can be obtained by showing that the gravitational energy given up in spreading the ring permits a ring thickness of only tens of metres to be sustained over the age of the Solar System. This implies that the maximum size particle in the power-law distribution is only a few metres in radius, similar to other estimates for uniform particle size cases[9,10]. This true thickness is, however, much smaller than has been inferred[1,2] from the ring-passage observations.

Dynamical perturbations of ring particles by the Sun and by Saturn's large satellites may be relevant to this discrepancy. Under the disturbing action of a single satellite along with the planetary oblateness, the pole of a particle's mean orbit plane can be found to precess at a nearly uniform rate and at a nearly constant inclination relative to a mean pole, that of the Laplace plane[13]; for a ring particle, because it moves near the very oblate Saturn, the Laplace plane is almost, but not quite, coincident with the planet's equatorial plane. Following the usual celestial mechanics terminology, the displacement normal to the ring plane is decomposed into terms of different periodicities: short period ($T \sim$ particle orbit period), long period ($T \sim$ orbital precession period, orbital period of perturber) and secular ($T \sim$ infinity). The short period effects due to satellite and solar perturbations have an amplitude $\sim (m_j/m_{♄})(a/a_j)^3 \sin I_j$ (ref. 14) where m_j, a_j, and I_j refer to mass, semimajor axis, and inclination of the jth perturber. These amplitudes are of the order of 1 m and are coherent. Long-period and secular perturbations due to both the satellites and the oblateness of Saturn have been considered numerically[4] and qualitatively[16] by Bobrov. The principal effect of the oblateness is to slew or precess the particle orbit planes. However, Bobrov's[16] amplitude of 300 m was taken from a numerical integration by Franklin[11], which did not include the planetary oblateness (F. A. Franklin, personal communication). Using a perturbation technique, we have obtained a closed form solution for the long-period and secular portion of the particle orbits' inclinations (measured relative to the Laplace plane) as perturbed by a satellite (or the Sun), including planetary oblateness and planetary precession[13]. Ignoring the latter effect, but including collisional damping, this forced inclination has constant amplitude $\omega_j \sin I_j(\omega_f + 2n_j)^{-1}$, and the forced precession of the orbit node is at the rate of $2n_j$, where n_j is the mean motion of the jth perturber, $\omega_j = Gm_j\alpha_j^2 B_1^{3/2}(\alpha_j)/(4na^3)$ is the precession rate of the particle's orbit plane due to the jth satellite with G the gravitational constant, $\alpha_j = \mathrm{a}/\mathrm{a}_j$, $B_1^{3/2}(\alpha_j)$ is a Laplace coefficient[13], and n is the particle's mean motion; $\omega_f = (3/2)J_2 n(r_0/a)^2$ is the precession rate of the particle's orbit plane due to the planetary oblateness represented by the gravitational coefficient J_2 as normalised to the saturnian radius r_0. It is the inclusion of the effects of oblateness, manifested in ω_f, that effectively reduces the amplitude of the satellite perturbation compared with previous results[4,11,16]. Typical amplitudes are less than a few metres due to the satellites, with Titan, Tethys, and Mimas being the only important ones, and $\sim$10 m for the response to the solar perturbation which has the same character; these amplitudes increase by about an order of magnitude from the inner to the outer edge of the ring. A similar and still coherent effect is produced by the precession of Saturn's equatorial plane under solar torques, whereby particles in the mean ring plane experience noninertial effects causing particle orbit inclinations to oscillate while following the precession cycle of Saturn.

The subsequent evolution of such 'coherent' perturbations, in particular whether they may be coupled into random motions of the ring particles, has long been debated. The nature of the mathematical solution given by Burns *et al.*[13] makes it fairly clear that there is no means by which this can occur. In contradiction to a previous suggestion[16], differential precession of orbits of particles having similar inclination and slightly different semimajor axes, is not a property of the forced solution which dominates in the presence of damping (energy loss in collisions), Such 'winding up' does occur for a particular set of initial orbits randomly distributed about the mean ring plane (see equation (71) of ref. 13). 'Initial' noncircular components (random velocities) are, however, damped out on the flattening time scale, and the solution relaxes to the fully forced case which is coherent and time-independent (see for example equations (67) and (68) of ref. 13).

The above mechanisms produce displacements from the mean ring plane that are much less than the nominal 'observed' ring thickness. However, the Laplace plane mentioned above, about which the several metre coherent displacements occur, is itself significantly inclined to Saturn's equator by an amount that depends on the relative strength of the gravitational influence of Saturn and the various perturbing satellites, and hence on the semimajor axis a of the particle orbit. Thus, the inclination of the mean ring plane varies with radial distance throughout the ring system, giving vertical displacements at the outer edges of the rings of about $\pm 10^2$ m each for perturbations due to Mimas, the Sun, and Titan, and smaller values for the other satellites. The nature of the result is a ring that is 'flipped' up and down at its opposite edges by possibly a total difference of several hundred metres, depending on the orbital parameters of the various perturbers. In addition, light scattered by the exterior, diaphanous E ring could also be misinterpreted as a 'thickness' of about 100 m or more[13]. Although the total effect is still much less than the 3 km 'upper limit' suggested by Lumme and Irvine[3], it is not so far from their formal 'best result' of 800 m.

In conclusion, the 'vertical' structure and thickness' of the rings encompass several distinct phenomena, produced by different causes and manifested in different observations. The quality that best describes the local microstructure of the rings is probably a true thickness of some tens of metres, caused by local, gravitationally induced, random particle motions; such a vertical structure is consistent with the opposition effect[4,14] and with microwave observations[6,7]. Satellite and solar perturbations produce two significantly different effects. First, coherent metre-scale vertical displacements, similar for all particles with similar orbital elements, are superposed on the local mean ring plane. There are both long-period and short-period displacements. The long-period displacements are characterised by particle orbits with a constant forced inclination and nodes that advance at twice the mean motion of the satellite perturber. The short-period displacements are tied to the longitude of the perturber. Second, the vertical displacement from the saturnian equatorial plane characterising the mean ring plane varies nonlinearly with radial distance and may produce an apparent edge-on thickness of several hundred metres, which would make it impossible for the true 'thickness' or local vertical scale height of the rings ever to be determined by ring-passage observations.

Our result—namely, that considerations of energy balance, combined with an R^{-3} power law with maximum radius $R_{\max}$, allow an approximate $R_{\max}$ of only several metres—has interesting implications for the origin of the rings. Particles that condensed directly from a protoplanetary nebula might be expected to be of this size, whereas residual particles resulting from Roche breakup of a large object would be expected to be significantly larger[16,17].

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Kinetics of electron attachment to oxygen and water in flames

J. M. Goodings* & A. N. Hayhurst

Department of Chemical Engineering and Fuel Technology, University of Sheffield, Mappin Street, Sheffield, UK

Negative ions are found in flames[1] and are presumed to arise from electrons attaching to some unidentified flame species, possibly molecular oxygen[2,3]. Free electrons and positive ions are produced in a flame either by chemi-ionisation[4] in $CH+O \rightarrow CHO^{+}+e^{-}$ or by collisional ionisation[5] of a metal, such as potassium, in $K+M \rightarrow K^{+}+e^{-}+M$, where M represents a third body. An atmospheric pressure flame is an interesting medium in which to measure the rate of appearance of negative ions; normally no more than one particle in 10^6 is electrically charged, there are usually no electric or magnetic fields and the attaching electrons are 'thermalised', that is, their energies are governed by the flame temperature. In addition, electron attachment is at much higher temperatures and pressures than normal[6,7] and in a system free from wall effects. We present here a study of the kinetics of electron attachment to oxygen and water in flames. The rate coefficients suggest a surprisingly large negative temperature coefficient.

Most of this work was done with an oxygen-rich atmospheric-pressure flame of unburnt composition: $H_2/O_2/N_2 = 1.5{:}1.0{:}4.67$ and temperature 1,810 K. The burner[8] gave a cylindrical flame (diameter 12.2 mm) with an almost flat reaction zone, followed by a burnt gas region in which there was laminar and plug flow, with conditions on the axis being unaffected by the surrounding atmosphere for around the first 25 mm. The mole fractions of the major constituents of the burnt gases were: $O_2 = 0.0379$; $H_2O = 0.233$; $N_2 = 0.726$. In addition, radicals were present with mole fractions for equilibrium in the burnt gases of: $H = 2.97 \times 10^{-6}$; $OH = 6.80 \times 10^{-4}$; $O = 2.42 \times 10^{-5}$; $HO_2 = 2.45 \times 10^{-7}$. Concentrations of H atoms were measured on a slightly different burner by an adaptation[9] of the Li/Na comparison method[10]. The results were expressed as a disequilibrium parameter γ_H ($=[H]/[H]_e$), that is, the extent by which hydrogen atoms exceed their equilibrium concentration $[H]_e$ for the almost constant temperature of the burnt gases. It can be shown that $\gamma_H^{1/3} = \gamma_{OH} = \gamma_O^{1/2}$. Values of γ_{OH} in this flame were ~8 close to the reaction zone and ~1.4 at 20 mm downstream, after a time elapse of 3 ms. Because the axial velocity (7.0 m s^{-1}) in the burnt gases is well defined, observations of ion concentrations at different distances enable kinetic measurements to be made.

Free electrons were produced by atomising an aqueous solution of KOH into the burner supplies. Ion concentrations were measured, as previously described[11,12], by continuous sampling from points along the burnt gases into a quadrupole mass spectrometer. The major ions were K^+, $K^+.H_2O$, OH^-, $OH^-.H_2O$ and O_2^-. Their abundances along the flame are given in Fig. 1, where the concentrations of hydrates have been included in those of the parent ions, because hydrates are largely formed during sampling[13]. Charge neutrality enables the electron concentration to be derived from $[e^-] = [K^+] - [Y^-]$, with $[Y^-] = [OH^-] + [O_2^-]$, that is, the total concentration of negative ions. Figure 1 shows that $[e^-]$ and $[K^+]$ rise much more rapidly than $[OH^-]$. This is consistent with K atoms undergoing collisional ionisation to give K^+ and free electrons, followed by electron attachment yielding O_2^- and OH^-. The ratio $[OH^-]/[O_2^-]$ increases with distance to a steady level above that calculated for equilibrium. That the ratio initially rises suggests

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*Present address: Department of Chemistry, York University, 4700 Keele Street, Downsview, Ontario, Canada M3J 1P3.

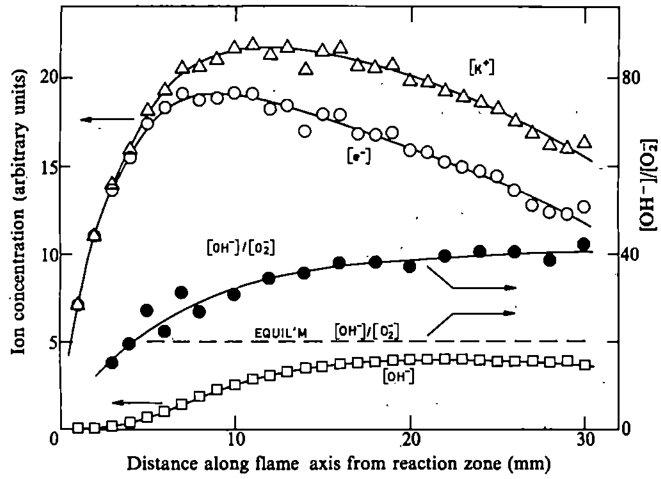

Fig. 1 Ion concentrations along the axis of the flame described in the text with 3×10^{-3} M KOH being sprayed in the atomiser. Also shown is the observed $[OH^-]/[O_2^-]$, together with its equilibrium value of 20.1 (dashed line).

that OH^- is produced from O_2^-. The near constancy of the ratio indicates that some rapid reaction couples the concentrations of OH^- and O_2^-, coming close to a local balance or equilibrium after a few millimetres. In contrast, the ratios of either $[OH^-]$ or $[O_2^-]$ to $[e^-]$ are not constant, being smaller than their equilibrium values, which in fact are approached well downstream. All this indicates that electron attachment giving O_2^- and OH^- is slow enough here to be a kinetic, rather than a steady-state, situation.

The possible formation and loss reactions for OH^- and O_2^- are:

$$
\begin{array}{rll}
e^- + O_2 + M \rightleftarrows O_2^- + M & (1) & k_1 = 1\times10^{-30} \\
O_2^- + OH \rightleftarrows OH^- + O_2 & (2) & \\
e^- + OH + M \rightleftarrows OH^- + M & (3) & k_3 = 3\times10^{-31} \\
e^- + H_2O \rightleftarrows OH^- + H & (4) & k_4 = 4\times10^{-17} \\
 & & k_{-4} = 1\times10^{-11} \\
e^- + HO_2 \rightleftarrows OH^- + O & (5) & k_{-5}(298\text{ K}) = 2\times10^{-10} \\
e^- + H_2O_2 \rightleftarrows OH^- + OH & (6) &
\end{array}
$$

where k_x and k_{-x} are the rate constants of the forward and reverse steps of reaction (x), whose equilibrium constant $K_x = k_x/k_{-x}$. The quoted values are those recommended by Jensen and Jones[14] for 1,810 K in the ml molecule^{-1} s^{-1} system of units. Reaction (3) can be discounted in our flame, as not being competitive with (1); similarly, step (−3) is much slower than (−1) because the electron affinity of OH exceeds that of O_2. It might be noted that reaction (3) is the sum of (1) and (2), so that $K_3 = K_1K_2$. The value given for k_{-5} refers to room temperature[15]; k_{-6} has not been measured, although steric effects might be expected to make it smaller than k_{-4} and k_{-5}, for which the neutral reagent is atomic. Reaction (2) is the most likely interconversion process for OH^- and O_2^-, in which case Fig. 1 indicates it is close to being balanced a few mm from the reaction zone. Also, the initial rise in $[OH^-]/[O_2^-]$ is consistent with O_2^- being the first negative ion formed; that this ratio exceeds its equilibrium value is because $\gamma_{OH} > 1$ and, more importantly, reaction (2) is fast enough to proceed in the exothermic direction during sampling[8]. This latter conclusion is confirmed by the fact that the observed $[OH^-]/[O_2^-]$ depended slightly on the diameter of the sampling orifice. This compares with the ratio $[e^-]/[Y^-]$ being independent of hole size, in line with a relatively slow production of negative ions Y^- by electron attachment.

As a first step, consider only reactions (1) and (2), so that:

$$d[Y^-]/dt = k_1[e^-][O_2][M] - k_{-1}[O_2^-][M]. \qquad (I)$$

This may be expressed as:

$$y = \frac{v}{[e^-]}\frac{d[Y^-]}{dz} = k_1[O_2][M] - \gamma_{OH}^{-1}\frac{[OH^-]}{[e^-]}\frac{k_1[O_2][M]}{K_3[OH]_e}, \qquad (II)$$

with the flame velocity $v = dz/dt$ and $[O_2^-] = [OH^-][O_2]/(K_2[OH]_e\gamma_{OH})$, provided (2) is balanced. All this is checked in Fig. 2*a*, which is a plot of y against $x_1 = \gamma_{OH}^{-1}[OH^-]/[e^-]$. For this, values of y were obtained by numerically smoothing and differentiating[16] the distance profile of $[Y^-]$. It is seen in Fig. 2*a* that y initially rises and is then linear in x_1 from 9 to 16 mm from the reaction zone. The central linear portion yields $k_1 = 3.2\times10^{-34}$ from its y intercept and the different $k_1 = 4.5\times10^{-34}$ ml^2 molecule^{-2} s^{-1} from the slope.

The departures from linearity are understandable in terms of minor contributions from reactions (4)–(6). The inclusion of reaction (4), along with (1) and (2), gives the rate equation:

$$y = k_1[O_2][M] + k_4[H_2O] - \frac{[OH^-]}{[e^-]}\times (\gamma_{OH}^{-1}k_1[O_2][M] + \gamma_{OH}^{3}k_4[H_2O])\frac{1}{K_3[OH]_e} \qquad (III)$$

where $[H]_e/K_4 = [H_2O]/(K_3[OH]_e)$. Letting $r = k_4[H_2O]/k_1[O_2][M]$ be the ratio of the forward rates of reactions (4) and (1), an estimate of r can be obtained from Fig. 2*a* where $y = 0$. This is at $z = 22$ mm from the reaction zone, where $\gamma_{OH} = 1.32$ and $[OH^-]/[e^-]$ has approximately its equilibrium value of $K_3[OH]_e = 0.250$. (This latter observation confirms that our measurements of $[OH^-]$ and $[e^-]$ are free from sampling falsifications[8,13].) Thus, equation (III) may be solved for the derived value of $r = 0.185$, whose magnitude shows electron attachment in (4) to be much less than via (1). In any case, equation (III) becomes:

$$y = (1+r)k_1[O_2][M] - (\gamma_{OH}^{-1} + r\gamma_{OH}^{3})\frac{[OH^-]}{[e^-]}\frac{k_1[O_2][M]}{K_3[OH]_e} \qquad (IV)$$

Figure 2*b* shows a plot of y against $x_2 = (\gamma_{OH}^{-1} + r\gamma_{OH}^{3})([OH^-]/[e^-])$ with $r = 0{:}185$. There is now a more extensive region of linearity from 9 to 24 mm, which yields $k_1 = 6.6\times10^{-34}$ from the y intercept and $k_1 = 6.4\times10^{-34}$ ml^2 molecule^{-2} s^{-1} from the slope, that is, differing by only 3%. The mean value of $k_1 = 6.5\times10^{-34}$ together with $r = 0.185$ gives $k_4 = 7.9\times10^{-17}$ ml molecule^{-1} s^{-1}, which is in excellent agreement with Jensen and Jones's estimate[14], based largely on a room temperature measurement[17] of k_{-4}. The occurrence of points in Fig. 2*b* off the straight line is thought to reflect slight inaccuracies in γ_{OH}, which appears as $\gamma_{OH}^{3} (= \gamma_H)$ in equations (III) and (IV), rather than deficiencies in the kinetic model. For example, the point

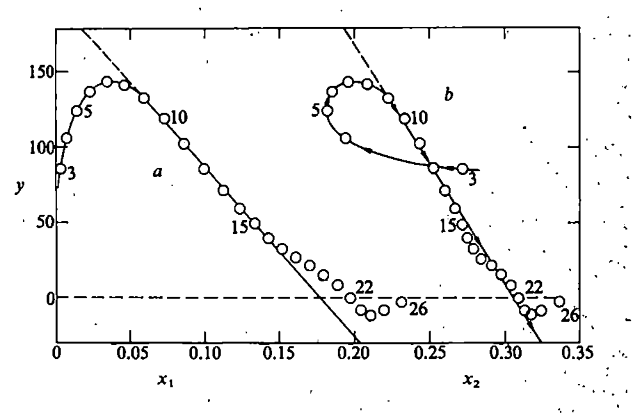

Fig. 2 The data in Fig. 1 plotted as $y = v/[e^-]\times d[Y^-]/dz$ against $x_1 = \gamma_{OH}^{-1}[OH^-]/[e^-]$ in *a* and against $x_2 = (\gamma_{OH}^{-1} + r\gamma_{OH}^{3})[OH^-]/[e^-]$ in *b*. Points are numbered according to their distance from the reaction zone in mm and the arrows in *b* denote the sequence of points at increasing times in the burnt gases.

5 mm from the reaction zone becomes on the straight line, if γ_{OH} there is changed from 2.84 to 3.08, which is just within the error bounds for γ_{OH}. Figure 2*b* shows that *y* is initially small, then increases and finally becomes negative. That $[Y^-]$ first shifts away from and then back through equilibrium is mainly caused by γ_{OH} declining, and consequently electron detachment being initially dominated by (−4), then becoming less important and finally increasingly by (−1).

A detailed examination shows that the effect of including process (5) is to shift those experimental points for conditions near the reaction zone farther away from the straight line in Fig. 2*b*. We thus conclude that the contribution from reaction (5) is insignificant. Moreover, similar reasoning applied to reaction (6) indicates negligible participation, particularly because $[H_2O_2]_e$ is extremely small at flame temperatures. Finally, the derived k_1 and k_4 were found not to depend systematically on either the diameter of the sampling orifice or on the amount of potassium added to the flame. This establishes that our measurements are free from any falsifying perturbations encountered[8,18] with fast reactions proceeding during sampling.

In conclusion, we find $k_4 = 8 \pm 4 \times 10^{-17}$ ml molecule^{-1} s^{-1}, which is very much in line with estimates based on room temperature data[14,17]. Also, the behaviour of $[OH^-]/[O_2^-]$ in Fig. 1 is consistent with $k_2 > 10^{-12}$ ml molecule^{-1} s^{-1}, which is not surprising. On the other hand, our conclusion that $k_1 = 6 \pm 3 \times 10^{-34}$ ml^2 molecule^{-2} s^{-1} is much less than the lower temperature measurements of $k_1 \sim 10^{-31} - 10^{-30}$ for a reaction not expected to be very temperature-dependent[6,7,14]. That reaction (3) is not participating indicates that k_3 must also be much smaller than the estimated[14] value of 3×10^{-31} at ~2,000 K. We can only conclude that three-body electron attachment in a process like (1) or (3) has a surprisingly large negative temperature coefficient.

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Combined rotating and linear motion effects on the wear of polymers

B. J. Briscoe & T. A. Stolarski

Department of Chemical Engineering and Chemical Technology,
Imperial College, Prince Consort Road, London SW7, UK

The unusually low friction of polytetrafluoroethylene, PFTE, is utilised in many commercial applications[1]. Unfortunately this low friction is often accompanied by rather high wear, and it is generally necessary to modify the polymer, usually by the incorporation of solid inorganic filler particles, to reduce the wear to acceptable levels[1,2]. Although several attempts have been made to establish the mechanism of this improved wear behaviour, the optimisation of filler type, size and concentration remains an empirical exercise, and several relatively simple wear testing machines have been developed to assess prospective materials[3,4]. These tests attempt to reproduce the sliding conditions encountered in practice. A widely used test is the pin-on-disk configuration where a fixed pin of polymer is loaded against a rotating disk. The load and relative velocity are fixed and the pin describes a circular path over the disk surface for a predetermined period, after which the wear is measured, generally by weighing the polymer pin. Important additional variables are the environment, the surface topography of the disk, the temperature and the geometry of the pin[5]. In the process of conducting such a series of measurements we have identified and report here an additional variable which may be of general importance: the radius, *R*, of the path described by the pin on the disk.

Figure 1 shows data for the wear per unit distance, *K*, for specimens of PTFE and low density polyethylene, LDPE, as a function of *R* obtained using a pin-on-disk machine (for experimental details see ref. 6). We observe that *K* is a marked function of *R* for PTFE but not for LDPE. Figure 2 shows the same data and also data for other polymers as well as carbon-filled PTFE. Here, the wear data have been normalised by dividing the $K(R)$ obtained at a particular value of *R* by the value for $K(\infty)$ which is obtained by extrapolating $K(R)$ against $1/R$ curves to $1/R = 0$. The value of $K(\infty)$ corresponds to the wear rate for 'pure' linear sliding. In Fig. 2 the data are presented as the ratio of $K(R)/K(\infty)$ against $1/R$ which has the virtue of superimposing the sets of data for PTFE and linear polythene on two master curves. The sliding velocities, *V*, and nominal contact pressures are constant for each specimen. The disk is cooled by a coolant flow beneath the disk surface and the bulk temperature is ~20 °C (ref. 6). Note that two polymers, polymethylmethacrylate, PMMA, and LDPE, show no radius effect until *R* approaches the radius of the pin. However, for PTFE and a carbon-filled PTFE composite the wear decreases significantly as *R* is decreased. The data for high density polythene, HDPE, and ultrahigh molecular weight polythene, UHMPE, show a similar but less marked effect. Simultaneous measurements of the frictional force have also been made, and these data indicate that where the wear decreases the friction increases.

The common reduction in wear as *R* approaches the pin radius is due to the fact that, although the polymer surface is damaged, the comparatively small relative displacement of the surfaces enables much of the wear debris to be confined in the contact zone. The weight loss from the pin is thus reduced. At larger values of *R* the unusual behaviour of PTFE and, to a lesser extent, the linear polythenes seem to arise from a peculiarity in the pin-on-disk method. Since the pin describes a circular path the motion is complex and may be considered to be comprised of a linear vector, *V*, combined with a pin rotating with an effective angular velocity, ω_p, equal to that of the disk, ω_d. As *R* decreases while *V* is maintained constant, ω_p increases as $1/R$.

It is this angular component which we believe is responsible for the unusual friction and wear behaviour shown by PTFE and the linear polythenes. The reason for this effect is not clear, but the following rationale accounts for the results. PTFE and HDPE have unusual friction and transfer behaviour although the behaviour is more pronounced by PTFE[7,8]. If a PTFE pin is slid linearly at low speeds against a clean smooth surface, the static frictional force is initially high and a relatively thick transfer layer is deposited. Further sliding produces a thinner and more highly orientated film and a lower dynamic frictional force. If sliding is arrested and the polymer pin is then rotated about its load axis by 90°, the initial behaviour is repeated[7]. We have also conducted a series of measurements where polymer spheres of ~8 mm diameter have been subjected to a simul-

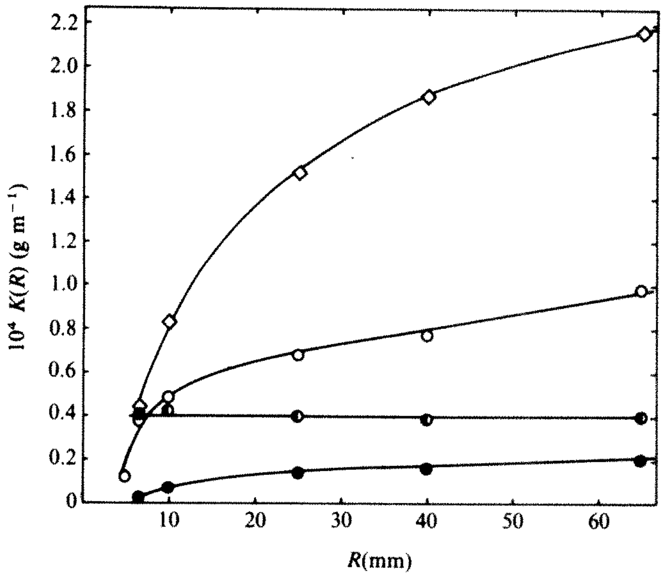

Fig. 1 Rate of wear, $K(R)$, expressed as mass loss per unit sliding distance, as a function of R, the radius of the wear path for PTFE and LDPE. Sliding velocity: 0.5 m s^{-1}; nominal contact pressure: 6×10^5 Pa; counterface temperature ~20 °C; counterface mild steel, roughness ~0.1 μm c.l.a.; pin diameters: PTFE ●, 6 mm; ○, 13 mm; ◇, 20 mm; LDPE ◐, 13 mm (wear rate × 10^2). The wear rate is a marked function of R.

taneous combination of linear and rotating motion in an attempt to reproduce the effective motion of the pin in the wear testing machine. The frictional force in the direction of linear motion was found to be extremely sensitive to the introduction of rotation for PTFE materials and also to a lesser extent for HDPE and UHMPE. The frictional force in this direction typically increases from the low kinetic value to a maximum value close to the static value as the imposed rotational velocity is increased, and it reaches a maximum when the angular velocity is such that the contact area is rotated by $\pi/2$ for a linear travel approximately equal to the contact diameter. This result is consistent with the response when a linear motion is interrupted and an instantaneous $\pi/2$ motion is imposed on the contact. When repeated traversals of the fixed polymer pin are made over the transferred layer, the pin seems to displace the layer readily and a further layer is deposited. This process leads to high wear and there is evidence that the rate of wear is greatly influenced by the magnitude of the adhesion of the transferred layer to the substrate[8,9]; the greater the strength of adhesion the lower the rate of wear. When rotation is introduced during linear sliding the transferred films seem to be somewhat thicker and more tenaciously attached to the counterface for PTFE, although we have no quantitative results. These effects are restricted to sliding on smooth counterfaces and apparently to contacts where either the sliding velocity or the contact pressures have moderate values. For a large apparent contact pressure of ~1.3×10^7 Pa the unusual response decays quickly at sliding velocities in >20 mm s^{-1}. At a more moderate contact pressure of 6×10^5 Pa, which is typical of commercial contacts, the unusual behaviour is maintained to above 2.0 m s^{-1}. When the counterface is rough, for example when the centre line average roughness is ~3.0 μm, the unusual friction behaviour of PTFE is absent in all the contact conditions studied.

In contrast LDPE shows only high friction and thick film behaviour with no orientation effects. This polymer also shows no increase in the frictional force as measured in the linear sliding direction when rotation is imposed. PMMA is similarly insensitive. The special friction and wear behaviour seems to be restricted to 'smooth molecular profile' polymers when they are slid on smooth counterfaces and in no more than moderately intense contact conditions. These effects seem to be associated with reorientation processes occurring in the contact. The introduction of rotation into a linear sliding motion will clearly affect this process and hence produce thicker transferred films. The friction data observed in the pin-on-disk experiment are consistent with this, as are the low speed data. To explain the reduction in wear with small additions of angular velocity we must then assume that the thick unorientated layers adhere more strongly to the substrate than the thinner more highly orientated films[7] and hence a lower wear results. The comparatively low wear of LDPE compared with PTFE and HDPE in comparable conditions is consistent with this hypothesis.

The results on the pin-on-disk machine indicate that the radius effect described may be interpreted if we assume that the polymer pin is effectively rotated as it describes its circular path of radius R. We have conducted a series of measurements where the pin is actually rotated about its load axis while it is in contact with the rotating disk. The direction and magnitude of this pin rotation, ω_p, have been varied over a wide range for a fixed value of R. When the value of ω_p is equal in magnitude to ω_d but rotating in the opposite direction the wear rate of PTFE is observed to have its maximum value. This corresponds to effectively 'pure' linear sliding. If, however, the pin is rotated in the same direction as that effectively induced by the disk's rotation a minimum in the wear rate is observed when ω_p is such that the total pin rotation is equal to a value of $\pi/2$ for a linear travel distance of approximately one contact diameter. For the contact conditions studied (contact pressure 1.5×10^6 Pa, sliding velocity 0.2 m s^{-1}) there is a factor of about 3.5 difference between the maximum and minimum values of these wear rates. Between the two extremes there is a gradual decrease in wear as the amount of rotation is increased. When the amount of rotation is further increased beyond the minimum point the rate of wear increases. We have also observed a significant difference in the nature of the transferred film and the wear debris in these experiments. When the wear is at its maximum the transferred film is thin and the wear debris is highly fibrillar in appearance. At the minimum rate of wear the transferred film is thicker and the wear debris is in the form of the plates. In contrast the rate of wear and the appearance of the transferred layers and wear debris are insensitive to the introduction of imposed rotation for LDPE in similar conditions. These results confirm that the unusual behaviour of PTFE in the simple pin-on-disk experiment probably arises from the introduction of apparent rotation.

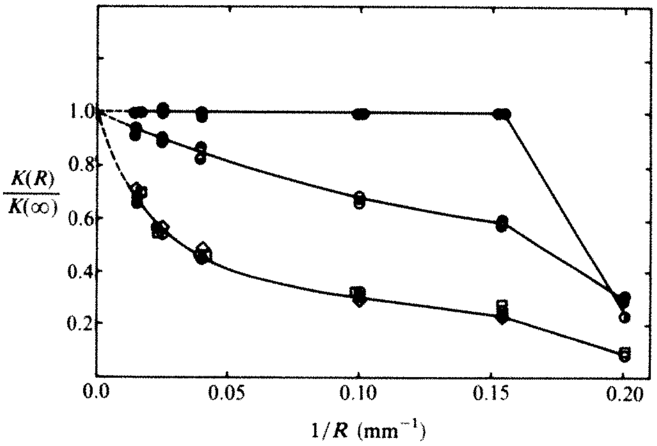

Fig. 2 Normalised wear rate, expressed as $K(R)/K(\infty)$ against $1/R$ where $K(\infty)$ is the estimated wear rate for linear sliding and R the wear track radius. Sliding conditions as for Fig. 1. □, Carbon-filled PTFE 1:5 w/w; ◒, UHMPE; ◑, PMMA; ◓, HDPE; test pin diameters, 13 mm. Other symbols as for Fig. 1. Polymers of the type HDPE, UHMPE and PTFE show a marked reduction in wear as R decreases, while PMMA and LDPE show no effect until R approaches the radius of the test specimen. For PTFE the reduction in wear is of the order of 70% while for HDPE and UHMPE the effect is more modest, approximately 40%. Further reductions are noted when the wear track radius decreases below that of the radius of the pin.

They further emphasise the peculiar sensitivity of this polymer to rotation during sliding and indicate that these effects arise from the disruption of natural orientation processes which are produced during sliding.

Our results have two consequences: at a practical level they indicate the need for caution when measuring the friction and wear of PTFE and, to some extent, HDPE and UHMPE and their composites using the pin-on-disk configuration, and from an academic point of view it introduces a new and interesting variable for probing the friction and wear mechanism of these materials.

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First observation of the coil–globule transition in a single polymer chain

Izumi Nishio, Shao-Tang Sun, Gerald Swislow & Toyoichi Tanaka*

Department of Physics and Center for Materials Science and Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

The conformations of single polymer chains in solution have been studied extensively since the 1940s. At high temperatures and in good solvents, a polymer has an extended coil configuration, while at low temperatures and in poor solvents a polymer is in a collapsed globule state. The transition between the extended and collapsed state as the temperature or solvent composition is varied was thought to be smooth and continuous, and experiments supported this idea[1]. However, in the 1960s it was suggested[2–4] that the transition between the two configurations, the coil–globule transition, was discrete. We now report the first observations of the single polymer coil–globule transition. The observations were made on polyacrylamide molecules dissolved in acetone–water mixtures.

The mechanism of the transition, as described by Lifshitz[4], is quite similar to that of the gas–liquid transition. The extended polymer corresponds to the gas state, whereas the collapsed polymer corresponds to the liquid state. Many others have contributed to the theory of the coil–globule transition[5–9]. For example, de Gennes[9], using the analogy of the magnetic transition, pointed out that the coil–globule transition of an infinitely long polymer may correspond to a tricritical point. On the experimental side, there have been reports on the temperature dependence of the radius of gyration of polystyrene in cyclohexane[10–13], and on polyacrylic acid in dioxane[13]. In these experiments, however, the existence of the coil–globule transition was not confirmed.

Recently, Tanaka[14] and Fillmore and Tanaka[15] observed the reversible collapse of a covalently crosslinked polyacrylamide network immersed in an acetone–water mixture. The collapse occurred upon changing either the temperature or acetone concentration of the system. The experiments showed that the volume of the gel can change reversibly by a factor of 100 at the transition. This suggests that a single polyacrylamide chain may undergo a coil–globule transition in acetone–water mixtures.

Acetone is a poor solvent for polyacrylamide, whereas water is a good solvent for the molecule. By gradually changing the composition of the mixture, the polyacrylamide molecules can be brought through the transition between the regions of good solvent and of poor solvent. In our experiment, we used monodisperse polyacrylamide polymers of molecular weight $5–6 \times 10^6$ (Polysciences Inc). A single chain of the polymer consists of approximately 8×10^4 acrylamide monomers and has a length of about 24 μm. The concentration of the polymer in solution was less than $10\,\mu\text{g ml}^{-1}$. At this concentration, the mean distance between adjacent polymers, of the order of 1 μm, was much larger than the average polymer size, thus interpolymer entanglements were avoided. An argon-ion laser (Spectra Physics Model 165) at 5,145 Å wavelength was used as a light source. The laser power was approximately 1 W. The correlation function of light scattered from the sample at a 90° angle was measured using a photomultiplier tube and a photon correlator (Nicoli Instruments). All measurements were made at 25 °C. From the decay rate of the correlation function of the scattered light intensity, we determined the diffusion coefficient, D, of the polymer, which is related to the hydrodynamic radius, ξ, of the polymer through the Stokes–Einstein relation[16]:

$$D = kT/(6\pi\eta\xi)$$

where k is the Boltzmann constant, T is the absolute temperature, and η is the viscosity of the solvent.

Figure 1 shows the hydrodynamic radius of the polymer plotted as a function of acetone concentration (open circles). At low acetone concentrations, the radius is large, approximately 500 Å. Near an acetone concentration of 39%, the polymer shows a drastic decrease in hydrodynamic radius to about

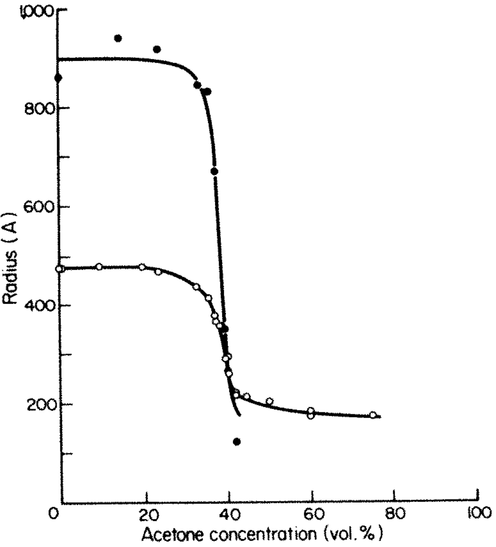

Fig. 1 The hydrodynamic radius of a single polyacrylamide chain, determined by laser light scattering spectroscopy, is plotted (○) as a function of the acetone concentration of the acetone–water mixtures used as solvent. The radius of gyration of the polyacrylamide chain, determined from dissymmetry measurements of the scattered light intensity, is also plotted (●). It was very difficult to determine the radius of gyration for values below 300 Å. Samples were prepared as follows. Polyacrylamide molecules were carefully dissolved in pure water, and gently stirred using a magnetic stirrer. The solution was then left standing for several days to ensure complete dissolution, before adding the solution to the acetone–water mixtures. The samples were then filtered through a 0.4-μm filter before the measurements were made.

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* To whom reprint requests should be addressed.

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200 Å. With further increases in acetone concentration, the polymer radius remains constant. We found the transition to be reversible and reproducible.

We also determined the radius of gyration of the chain from the angular dissymmetry of the scattered light intensity[17]. The results are shown in Fig. 1 (solid circles). The coil–globule transition is seen to occur at exactly the same acetone concentration as that seen in the curve of the hydrodynamic radius. The value for the radius of gyration, r_g, is about twice that of the hydrodynamic radius, ξ, in the coil state for the following reasons. First, for chain polymers, the hydrodynamic radius may be smaller than the radius of gyration due to such factors as the nature of the side groups on the polymer chain and the viscosity of the solvent. Measurements on polystyrene in solution[18] have shown ξ to be at least 20% smaller than r_g. The difference would be expected to be even larger for polyacrylamide, as it has much smaller side groups. Second, in our experiments, when r_g becomes higher than 500 Å, the fluctuations in the chain conformation begin to scatter light substantially[19,20]. This makes the apparent diffusion coefficient, calculated assuming that the scattering light intensity fluctuates due only to the translational motion of the polymer, larger than its actual value. Thus, the apparent ξ becomes less than the actual value. It is of interest to investigate further the amplitude and relaxation times of such chain configuration fluctuations.

The observed transition between the coil and globule states of the polymer is quite sharp, but not absolutely discrete. The finite transition width is probably due to the polymer chain comprising only a finite number of monomer units. A chain composed of an infinite number of monomers would be required to observe a discrete transition[4]. It will be important to investigate further the effect the finite size of the system has on the transition.

Thus we have presented direct evidence of the existence of the coil–globule transition in single polymers. The hydrodynamic radius of polyacrylamide chains in solution changes sharply in the transition between the regions of good solvent and of poor solvent. This sharp change in polymer size may function in amplification, switching, and memory at the molecular level and could have potential applications in biology, medicine and chemical engineering.

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Basaltic pillars in collapsed lava-pools on the deep ocean-floor

J. Francheteau*, T. Juteau† & C. Rangan‡

* Centre Océanologique de Bretagne, BP 337, 29273, Brest Cedex
† Université Louis Pasteur, Strasbourg
‡ Université Pierre et Marie Curie, Paris, France

Observations of peculiar volcanic objects, made by a submersible on the deep sea floor at a depth of about 2,600 m at and near the axis of the East Pacific Rise during the CYAMEX expedition as part of the RITA Project[1] are presented here. Two basic types of flow forms were observed within the crestal area of the East Pacific Rise: pillow flows and fluid lavas, the latter sometimes overlying massive flows. The East Pacific Rise at 21° N comprises an axial, unfaulted extrusion zone bordered by an extension zone characterised by faulting[2,3]. Pillow flows occupy the innermost or extrusion zone and constitute small elongated volcanic highs. Fluid lavas tend to occur at the edge of the adjacent extension zone in bathymetric lows controlled by normal faults or steep primary slopes of constructional highs. In the 50 × 200 m lows which border the extrusion zone the fluid lava is smooth and lobate surfaces which represent the upper surface of the flow are locally collapsed and reveal the internal structure of the fluid lavas. Where the roof collapse is extensive, layered columnar features are visible and volcanic layering can be seen against the flank of the bordering volcanic highs (Figs 1–3). Similar features have been reported from the Galapagos Rift[4]. The diameter of the approximately cylindrical pillars ranges from 0.5 to 2 m. Some pillars are made of multiple coalescent cylinders. The tops of the pillars are glassy, funnel-shaped and always widening upwards. The pillars were presumed to be hollow from several observations of gashes or openings in the vertical walls of the pillars. This was demonstrated during dive CY 78–19 to the south where a small pillar was toppled by CYANA and subsequent examination revealed a circular canal along the axis of the pillar. The outer surface of the pillars is marked by centimetre-thick glassy, subhorizontal ledges extending several centimetres from the outer vertical surface of the pillars (Figs 2–4). The ledges are spaced every 2–5 cm and show small lava stalactites hanging on the underside of the ledges. Examination of large layered fragments of pillars recovered by CYANA demonstrated that the layering is only a surface feature as it does not extend through the basaltic mass of the pillars. The apparent layering is due to glass ledges adhering to a vertical basaltic pipe. In some rare instances the pillar outer surface showed no ledges and instead a smooth surface corrugated with vertical grooves. Some pillars are inclined or slightly curved; others get narrower towards the base. The pillars are almost totally aphyric and have the same bulk composition as other lava types recovered in the axial zone of the East Pacific Rise at 21° N (ref. 5).

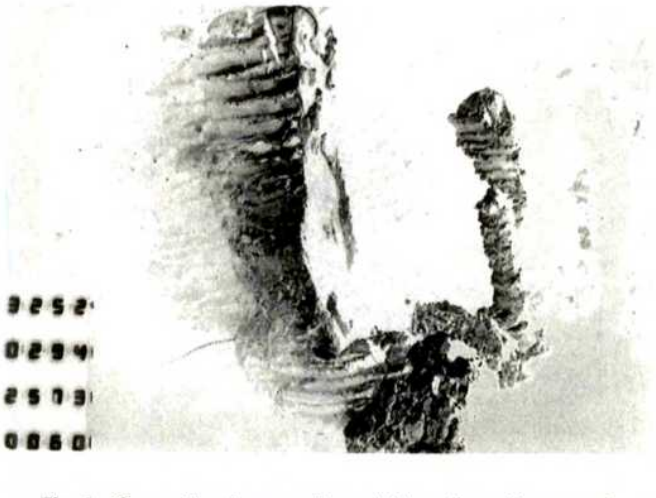

Fig. 1 General landscape: pillars within collapsed lava pond.

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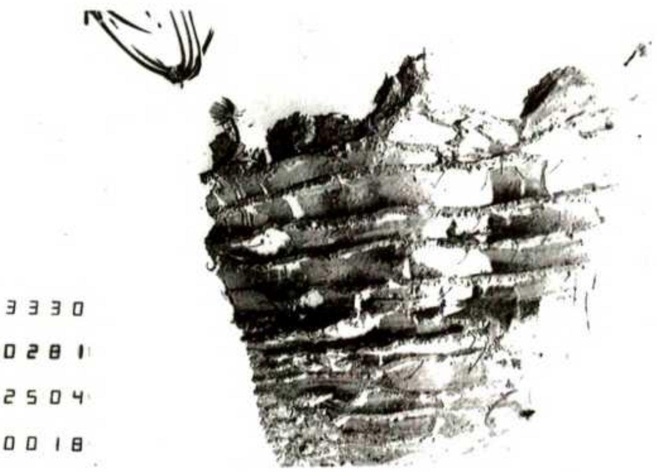

Fig. 2 Detail of a pillar showing the centimetric pseudo layering, with thin and darker salient glass layers projecting from the basaltic surface of the pillar.

The pillars occur in forest-like arrangement (Fig. 3). Their spacing ranges from 0.5 to 3 m and sometimes they coal c, building walls, especially near the edges of the depressions. However, the pillars are not confined to the edges. The flanks of the lows show large segments of undisrupted glass ledges analogous to those observed on the pillar surfaces. In one case (dive CY 78–19) where the fluid flows partially filled a graben, the layered lateral margin could be seen to represent a chilled, 5–10 cm-thick wall plated against the truncated pillow basalts of the normal fault scarp. The chilled margin was partially detached from the scarp surface.

Non-collapsed glassy upper surfaces of fluid lavas surrounding the fields of pillars show that in the depressions, the thickness of the lobate fluid lava roof is about 10 cm. The horizontal dimension of the lobes is of the same order as the pillar spacing and pillars provide roof support at the junction between adjacent lobes. The geometry of the lobate roof junction is analogous to that of the funnel-shaped pillar tops. Pieces of the lobate roof are commonly preserved around the fluid lava depressions and also where they form bridges between adjacent pillars. In most cases the lava pool appears to be concave upward with a slight (a few metres) depression in the middle of the pool with respect to the edges.

The seafloor around the pillars is strewn by rubble made up of pillar fragments and roof slabs. A deeply incised rille-like canyon was observed near the axis of the fluid–lava depression.

These observations lead us to the following interpretations (Figs 5 and 6):

(1) The fluid lavas fill a preexisting depression either controlled by faulting or by adjacent constructional highs. This is proved by inspection of the lateral contact and by the contrast in lava freshness between the fill and the adjacent pillows. The remarkable fluidity of the lavas cannot be ascribed to chemical differences or to temperature of extrusion. It probably reflects the large volume of outpouring which could in turn be a function of a very high spreading rate over a short time span.

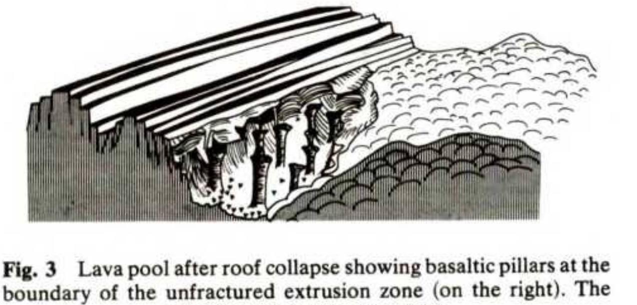

Fig. 3 Lava pool after roof collapse showing basaltic pillars at the boundary of the unfractured extrusion zone (on the right). The pillars are about 10–15 m high.

(2) The seawater which is trapped in the crust under the fluid lava pool is heated and expands (by a factor of 20 for a temperature of 800 °C at a depth of 2,600 m (see ref. 6), forcing its way up through the molten lava in a series of vertical conduits (J. Moore, personal communication) (Fig. 5). The walls of the conduits will be quenched at the boundary between the rising water and the hotter magma. The conduit walls will grow outward from the inner glassy walls of the conduits with time. This explains the hollow pillars found by CYANA. A similar explanation has been put forward by Fuller[7] and Waters[8] to account for the existence of vertical cavities (spiracles) that cut massive lava on the Columbia River Plateau. These authors proposed that water vapour and other gases surge upward into liquid lava that covers marshy ground.

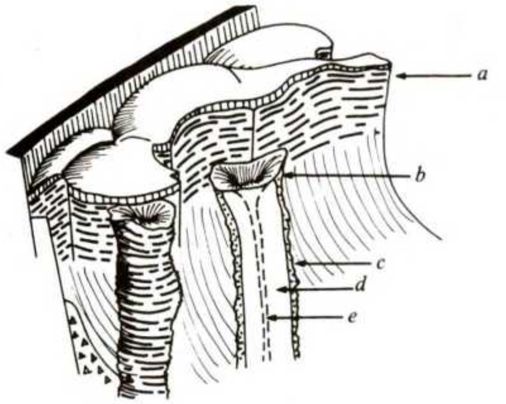

Fig. 4 Detail of basaltic pillar and of edge of magma pool. *a*, Pillar attached to the edge of the pool and capped by smooth lobate fluid lava. *b*, Pillar: funnel-shaped widening upwards Pillar cross-section showing: *c*, lava cooling ledges on outer margin; *d*, massive lava inside pillar wall; *e*, hollow conduit inside pillar.

(3) Before any extensive crystallisation takes place, the lava pool is drained rapidly in successive stages (Fig. 6). This leaves 'bath-tub' rings around the 'cold walls' present in the depression. This includes the pillars and the edges of the pool. This accounts for the apparent layering shown by the pillar samples. The rille-like canyon seen on the floor of the pool may be a drain path. Finally down-drag of either the roof or the outer rigid carapace of the pillars can scrape vertical grooves on the still plastic interior and explain the smooth corrugated outer surfaces of some pillars. A similar mechanism has been proposed by Moore and Richter[9] to explain the vertical striations on the surface of Hawaïan tree moulds.

(4) Solidification on the fluid lava against the cold wall at the bottom of the pool would help to maintain pillars in position after collapse of the roof.

Thus tall, near vertical and hollow pillars with rings of glassy ledges are found in dense arrangement within fossil pools of fluid lava. They are interpreted as the fossil witnesses of both

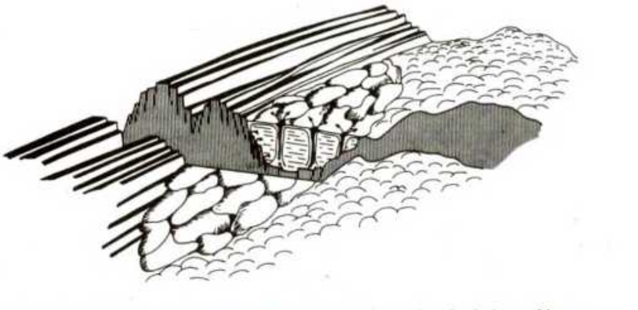

Fig. 5 Reconstruction of magma pool before the draining of lavas and roof collapse. The arrows at the pool surface represent escape of water.

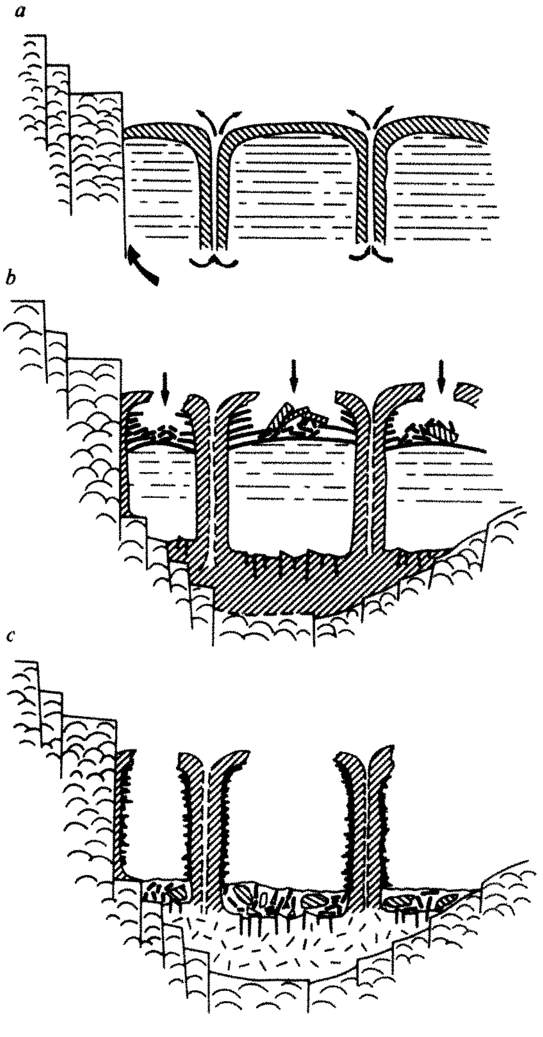

Fig. 6 Inferred evolution of magma pool showing history of pillars and cooling ledges at several stages (see text). *a*, Initial stage of filling of the depression; *b*, beginning of lava pool withdrawal with concomitant roof collapse. *c*, final stage of roof collapse showing isolated pillars standing among coarse rubble.

temporary liquid magma pools which can fill topographic lows and smoth the ridge topography, and of the rhythmic nature and rapidity of the magma pool withdrawal. When drain-off is prevented or when it proceeds too slowly, massive lavas are created.

The fluidity of the lava can be explained if there is rapid emplacement of a large volume of lava resulting in slow cooling of the bulk of the lava pool. The large volume of outpouring may be due to a high 'instantaneous' spreading rate concomitant with a high ascent rate of the lava.

The observations at 21° N show that the size of lava pools increases southward along the axis and thus that the fluid lava regime in the south is predominant over the pillow lava regime. This suggests that instantaneous rates of spreading may be variable along the strike of the mid-ocean ridge.

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Planktonic foraminiferal fauna associated with eastern Mediterranean Quaternary stagnations

Robert C. Thunell

Department of Geology, University of South Carolina, Columbia, South Carolina 29208

G. P. Lohmann

Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543

Bradley[1] predicted, before any evidence from sediment coring in the Mediterranena Sea, that changing late Quaternary climatic conditions would result in periodic stagnations of the eastern basin and with them, accumulations of organic-rich sediments. Such organic-rich layers from the eastern Mediterranean were subsequently observed in sediment cores by Kullenberg[2] and later named "sapropelitic layers"[3]. Since these initial observations, sapropels have been reported in most studies of Quaternary sediments from the Ionian Sea[4–14]. As a result, there is a great deal of information available concerning the chemical[15–18] and sedimentological properties[13–14], faunal characteristics[19] and temporal[9–11] and spatial[10,12] distribution of late Quaternary sapropels. In the study reported here we have attempted to characterise the planktonic foraminiferal biogeography of such late Quaternary sapropels in terms of the modern distribution of foraminefara in the Mediterranean Sea.

Sapropels are black, organic-rich muds with unusually high concentrations of hydrogen sulphide and iron hydroxide[15]. These sediments seem to be deposited in a particular sequence[13–14], reflecting the transition from open-marine sedimentation (hemipelagic) to that indicative of stagnant conditions. Sapropels are found throughout the entire eastern Mediterranean at depths greater than 800–1,000 m (ref. 12), although the areal extent of individual layers may not be basin-wide[10].

Distinct microfaunal and microfloral assemblages have been described from late Quaternary sapropels. Planktonic foraminiferal[5,9,10,19,21], nannoplankton[20], diatom[16] and pteropod[4] assemblages in sapropels are indicative of reduced surface salinities. For example, high frequencies of *Neogloboquadrina dutertrei*, a planktonic foraminifer preferring low surface salinities[22,24], are generally associated with late Quaternary sapropels[2,5,9–11,21]. Benthonic Formanifera and ostracodes, as well as any evidence of bioturbation, are totally absent from these layers[4,5,10].

Quaternary glacial–interglacial fluctuations have been a fundamental element of even the earliest theories of sapropel formation. Both Bradley[1] and Kullenberg[2] suggested that stagnation of the eastern Mediterranean resulted from lowered surface salinities produced by increased precipitation and runoff during glacial times. Subsequent studies[3,5,7,8,10,11] have also invoked a low salinity surface layer during sapropel formation, but demonstrated that this occurred during interglacial episodes. According to these studies, the flushing of freshwater from the Black Sea into the eastern Mediterranean, possibly combined with increased precipitation, forms a low salinity surface layer that prevents oxygenation of the deep basins and results in accumulation of anoxic sediments. The previously mentioned faunal evidence, together with recent studies on the oxygen isotopic composition of planktonic Foraminifera from sapropel layers, strongly support the existence of a low salinity surface layer[11,19,25].

The present study involves three basic steps: (1) characterisation of the sapropel-associated faunas; (2) comparison of these sapropel-associated faunas with the modern distribution of planktonic foraminifera; and (3) interpretation of the ancient sapropel environment in terms of the modern oceanographic conditions where faunas similar to those associated with sapropels are found. We have simply tried to find out where faunas most similar to the sapropel assemblage live in the modern Mediterranean and determine what is oceanographically unique about these environments.

The sapropel-associated planktonic foraminiferal assemblage was determined by averaging the faunas found in 12 late Quaternary sapropels from cores TR171-27 (33°50′ N, 25°59′ E) and TR172-22 (35°19′ N, 29°01′ E). The averaging process involves a Q-mode principal components analysis of all the foraminiferal frequency data obtained for the sapropel layers. The resulting first principal component (first eigenvector of the pseudo cos θ matrix[26]) represents the 'average sapropel fauna' for the late Quaternary in this region (Table 1). It accounts for $\sim$74% of the variance in the original data, with four species being particularly important (*Globigerinoides ruber*, *Neogloboquadrina dutertrei*, *Globigerina bulloides* and *Orbulina universa*). These species have been previously shown to generally increase in abundance within sapropel layers[5,7,9,10,21,27].

The present-day area distribution of this average sapropel fauna was found by comparing its composition with the planktonic foraminiferal compositions of 66 core top samples distributed throughout the Mediterranean. The core top faunal data, as well as other pertinent information on these samples, has been published previously[28]. The measure of similarity used is cos θ, where θ is the angle between the first principal component and a given sample. It is calculated simply as the product of the unit sample vectors and the unit eigenvector, and it varies from 0 to 1.

The area where core-top faunas are most similar to the average late Quaternary sapropel assemblage is shown in Fig. 1*a* (where similarities are greater than 0.8 on a cos θ scale of 0–1.0).

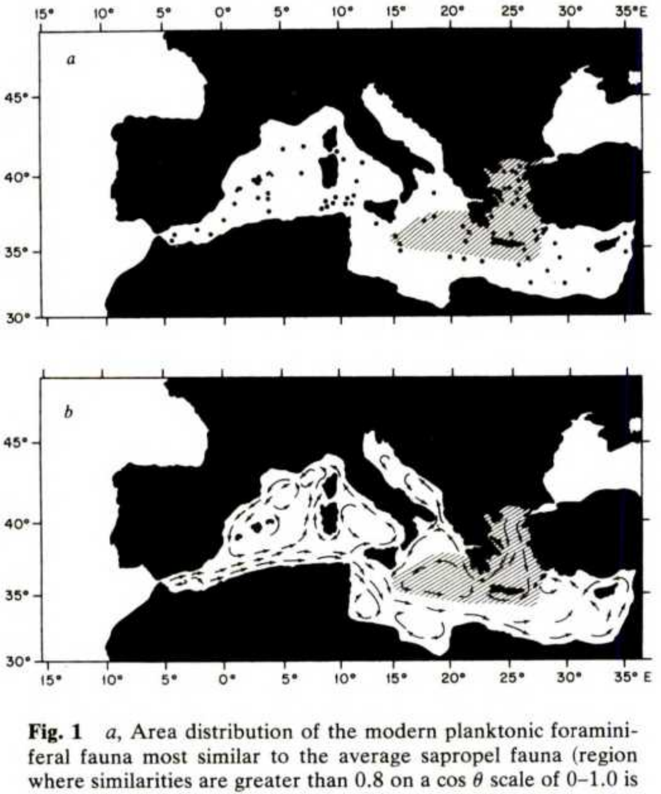

Fig. 1 *a*, Area distribution of the modern planktonic foraminiferal fauna most similar to the average sapropel fauna (region where similarities are greater than 0.8 on a cos θ scale of 0–1.0 is indicated). The core top faunal data is from ref. 28. *b*, Present day surface circulation of the Mediterranena presented by Lacombe and Tchernia[30]. The region containing the modern planktonic foraminiferal fauna most similar to the average sapropel fauna is indicated.

Table 1 Average sapropel fauna derived from a Q-mode principal components analysis of 18 planktonic foraminiferal species from 12 late Quaternary sapropel layers

Species	Per cent abundance
Globorotalia inflata	0.7
Globorotalia truncatulinoides (dextral)	0.0
Globorotalia truncatulinoides (sinistral)	0.5
Globorotalia scitula	1.6
Globigerinoides ruber	29.8
Globigerinoides sacculifera	1.1
Globigerinoides tenellus	1.3
Globigerina bulloides	18.6
Globigerina calida	0.0
Globigerina digitata	0.8
Globigerina falconensis	0.9
Globigerina quinqueloba	1.9
Globigerinella aequilateralis	3.6
Globigerinita glutinata	4.0
Neogloboquadrina dutertrei	24.3
Neogloboquadrina pachyderma (dextral)	1.9
Neogloboquadrina pachyderma (sinistral)	0.2
Orbulina universa	8.8

Abundances are loadings of species on the first eigenvector of the pseudo cos θ matrix[26] and are converted to per cent.

This region of maximum similarity includes the Aegean Sea and the area immediately west of Crete. A comparison of the area distribution of the modern fauna most similar to the average sapropel fauna with the present-day surface circulation of the Mediterranean reveals a close association with surface water that flows out of the Aegean Sea and forms a small gyre in the Ionian Sea (Fig. 1*b*). At present, the Aegean Sea receives a great deal of low salinity water from both river runoff and the Black Sea, with the salinity of the Black Sea water being $\sim$16‰ when it enters the Bosporus[28]. Although this low salinity water mixes rapidly within the Aegean, it is a component of the surface water that flows out of the Aegean. It is within the bounds of this Aegean-derived surface water that the modern planktonic foraminiferal fauna most similar to the average sapropel fauna is confined (Fig. 1*a*).

This biogeographic pattern may give further insight into the mechanism of sapropel formation. If the late Quaternary stagnations of the eastern Mediterranean are due primarily to the input of fresh water from the Black Sea, one would expect to find a low salinity lens extending from the Aegean over the remainder of the eastern basin during times of sapropel deposition. It seems that the biogeographic pattern resulting from a comparison of the modern fauna with the average sapropel fauna is simply a less severe version of what one would expect during periods of stagnation. That is, the present-day surface flow patterns in this region are analogous to those which were associated with times of stagnation. The fundamental difference between the two regimes is that during sapropel formation an intensification of this pattern occurs due to enhanced freshwater input. These results further support the idea that the Black Sea is the major source of freshwater needed to cause stagnation[3,5,19].

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Miocene tree shrews from the Indian Śivaliks

S. R. K. Chopra, S. Kaul & R. N. Vasishat

Department of Anthropology, Panjab University,
Chandigarh-160014, India

There have been no definite reports of fossil tree shrews although a rib cage from the Tatrot Formation of India has been identified as *Tupaia*[1], and fossil material, including the anterior part of a skull (YGSP 8089) with broken crowns of teeth (I^1–P^4) and a lower left molar (YGSP 8090), from the Middle Śivaliks of Pakistan, has been assigned to the sub-family Tupaiinae (unpublished results by I. Jacobs). The lack of material makes it difficult to assess the evolutionary position of this group, which, together with moles and hedgehogs, is considered to include the most direct modern descendents of the primitive placentals[2]. The living tree shrews have also attracted great interest because of the suggestion that they are related to primates. We report here a maxillary fragment (PUA I5) of the left side with M^1–M^3 and an isolated right lower molar, probably M^2, (PUA I6) recovered from the middle Śivalik deposits of Haritalyangar in Himachal Pradesh, India. We have referred this to the Tupaiinae, although we cannot assign generic and specific names. The following communication[3] describes more substantial material discovered later.

The maxillary fragment (Fig. 1*a*) consists of the molars M^1–M^3 and the posterior roots of premolar P^4. The molars, with the exception of the third, are quadrate in shape and decrease in size from front to back. Each tooth has three prominent cusps—paracone, metacone and protocone—which are connected by crests. The paracone and metacone are V-shaped, well-separated and near the middle of the tooth. Connected by the centrocrista comprising postparacrista and premetacrista, these two cusps form a W-shaped outer (buccal) wall. The disto-lingual aspect of M^1 has a small hypocone which is larger than that on M^2. The hypocone is low and near the neck of the tooth. The buccal portion of the crown of M^3 is broken, but in general this tooth is similar to the first two molars though much smaller. The metacone is also smaller and there is no hypocone on this tooth.

Buccal aspects of M^1 and M^2 display three small styles—parastyle, mesostyle and metastyle. The mesostyle on M^2 is larger than that on M^1. Because the roots are not visible in the maxillary fragment, nothing definite can be said about their nature and disposition. The wear on the occlusal surfaces is considerable.

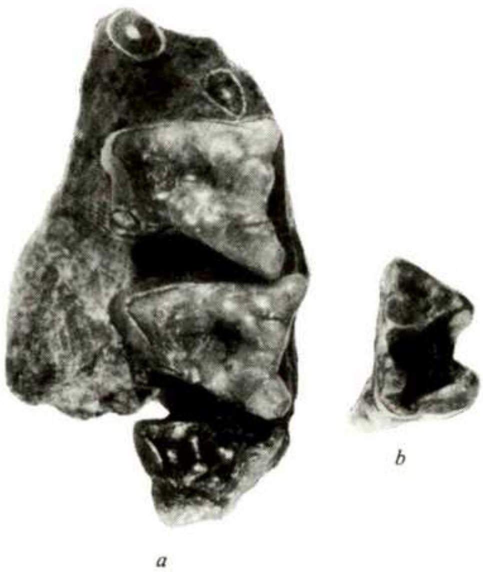

Fig. 1 Occlusal view of PUAI5 and PUAI6 (×7). (The characteristic features of the molar crowns are as identified in Fig. 2*a*.)

The isolated lower second molar of the right side (Fig. 1*b*) has the basic tribosphenic pattern with an elevated trigonid and a low talonid. Three prominent cusps—paraconid, metaconid and protoconid—constitute the trigonid. The talonid basin, which is larger than the trigonid, is bordered by the hypoconid, hypoconulid and entoconid. A moderately developed precingulum is present on the mesial aspect of the tooth. It is supported by two strong roots; the anterior one is broken almost halfway. The tooth shows slight wear due to use.

The maxillary fragment and the isolated M_2 (Fig. 1) have notable general similarities to those of the Tupaiinae (Fig. 2*a*). Like them, the upper molars lack internal cingula on the mesial

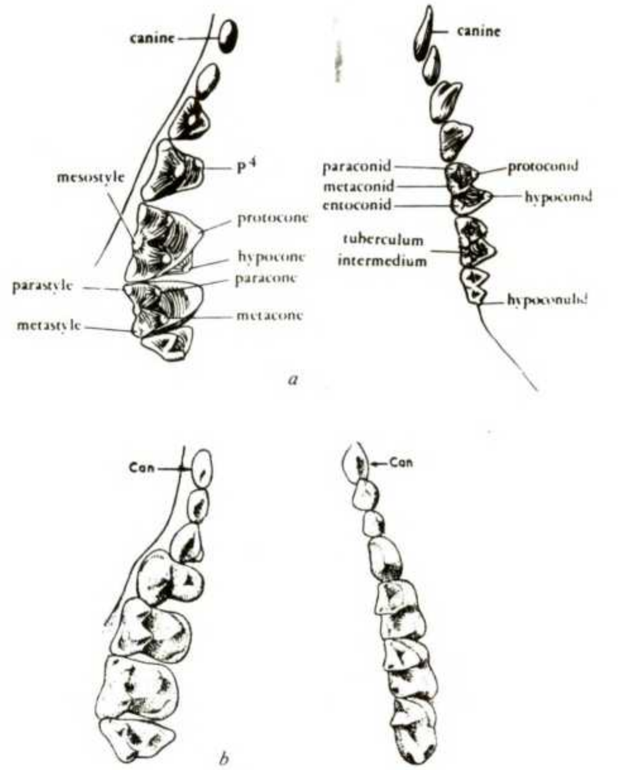

Fig. 2 Occlusal view of the left upper (*left*) and lower (*right*) teeth of *Tupaia glis* (*a*, ×2.2) and *Ptilocercus* (*b*, ×3.4). (*a* After Swindler[8], *b* after Clarke[4].)

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and distal aspects. The buccal cingulum is visible but the lingual cingulum is not. There is no tuberculum intermedium arising from the entoconid as it does in the lower molars of *Tupaia glis* (Fig. 2*a*). Like most members of the Tupaiidae, and unlike the Soricidae[5], M^1 and M^2 have distinct hypocones. Unlike those of *Ptilocercus* (Fig. 2*b*), the molars have a mesostyle and are not surrounded by a distinct cingulum on all sides[6–8].

A plausible taxonomic placing would need a more complete dentition than is available. However, similarities in morphology with the members of the Tupaiidae outweigh the differences, and, without assigning either generic or specific names at present, we believe that this material warrants its position in the sub-family Tupaiinae.

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Śivalik fossil tree shrew from Haritalyangar, India

S. R. K. Chopra & R. N. Vasishat

Department of Anthropology, Punjab University,
Chandigarh-160014, India

We report here a new taxon of fossil tree-shrew from the Śivalik deposits of Haritalyangar in Himachal Pradesh, India. There are no definitive records of fossil tree-shrews, for although two forms, *Anagale*[1] and *Adapisoriculus*[2] have been reported, their affinities with living tree shrews are disputed. Even the taxonomic position of the tree shrews is controversial, with some workers classifying them as primates[3–10] and others disagreeing. Butler[11] has suggested that they should be classified in a new order, Scandentia. The skull fragment (PUA I-3) described here is better preserved and much more complete than previous finds, and it has helped us to extend and confirm the identification of the specimens reported in the preceding communication[12].

Significant contributions to the understanding of anatomy, particularly of the skull and dentition of modern tree shrews have come from Clark Le Gros[5,6], Davis[13], Lyon[14] and Steele[15], and we have adopted the classification of Simpson[16] as follows:

Order	Primates
Superfamily	Tupaioidea
Family	Tupaiidae
Subfamily	Tupaiinae
Genus	*Palaeotupaia* new genus

Type: *Palaeotupaia sivalicus*, new species. [Named as an ancient form of the extant genus *Tupaia*, and after the Śivalik Hills where the fossil was found.]

Generic diagnosis: This is an ancestral and extinct form of the subfamily Tupaiinae with upper dentition 2.1.3.3. It resembles in some characteristics various members of the Tupaiinae, but resembles more closely *Tupaia* sp. in most anatomical details of skull and dentition. In size it resembles *T. minor*, but differs in having a proportionately longer face, less posterior incisive foramena, distinct protocones on P^3, more transverse P^4 and divided mesostyles. It differs from *Ptilocercus* (subfamily Ptilocercinae) in having a supraorbital foramen, well separated lacrimal and infraorbital foramena, lacrimal foramen in a distinct notch and well within the orbital margin and infraorbital foramen above P^3. It also differs in the position of the orbit in relation to the cheek teeth; in having large gaps between the central incisors–I^1 and I^2–and between I^2 and C, and in having a slight space between C, P^2 and P^3. P^3–I^1 is 1.7 × P^4–M^2; there is a large P^3; distinct mesostyles are present, and there is no distinct lingual cingulum or hypocone on the molars.

Palaeotupaia sivalicus new species

Type: PUA I-3 is a well preserved partial skull with left and right P^3–M^2 and roots and alveoli of central incisors, left lateral incisor, right canine, right M^3 and P^2. The right lateral incisor is unerrupted.

Hypodigm: Type plus a maxillary fragment (PUA I-5) of the left side with M^1–M^3, and an isolated right lower molar probably M^2 (PUA I-6) from Haritalyangar, Himachal Pradesh, India.

Horizon and locality: One and a half kilometres north-west of Haritalyangar, Bilaspur district, Nagri Formation,. Middle Śivaliks.

Specific diagnosis: Same as for the genus.

Description: The holotype (PUA I-3) under description is slightly damaged anteriorly. Posteriorly it is broken behind the level of left M^2 and behind the roots of M^3. Left M^2 is also chipped along its buccal margins. The palate appears to be fully ossified. The snout is comparatively long. The skull has a large orbit laterally (better preserved on the right side). Most of the cranial sutures are unclear. The maxillo-premaxillary suture arises from the posterior margin of the incisive foramen. The orbital surface of the maxilla is perforated by the apices of the lingual roots of M^1 and M^2. Ventrally and anteriorly, at the median transverse level of I^2 and behind the central incisors is a pair of large, oval incisive foramena. Along the lateral margin of the skull and immediately above the mesial root of P^3 is the anterior opening of a comparatively medium sized infraorbital foramen. A small foramen is present at the level of P^2. The lacrimal foramen is in a distinct notch which lies well within the orbital margin. At the dorsal surface of the skull there is a distinct supraorbital foramen, part of which is broken. The central incisors appear to be slightly larger than the lateral incisors and are separated from each other by a large gap. Similarly, I^2 is separated from I^1 by a comparatively larger gap. The single-rooted canine is set apart from I^2 by a large diastema. Although the small and single-rooted P^4 is very near to P^3 and C, the gap between the canine and P^2 is bigger than that between P^2

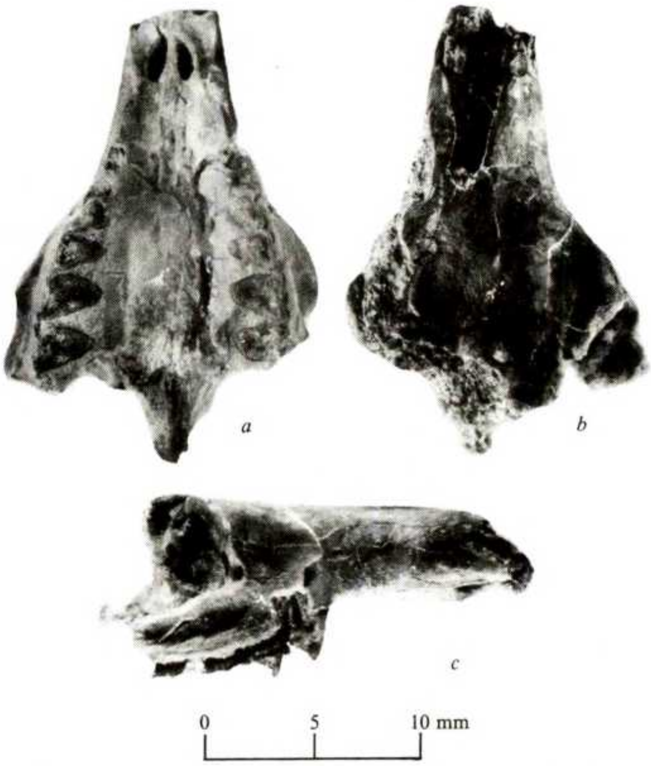

Fig. 1 *Palaeotupaia sivalicus* PUA I-3. *a*, Ventral view of the skull; *b*, dorsal view of the skull; *c*, lateral view of the skull.

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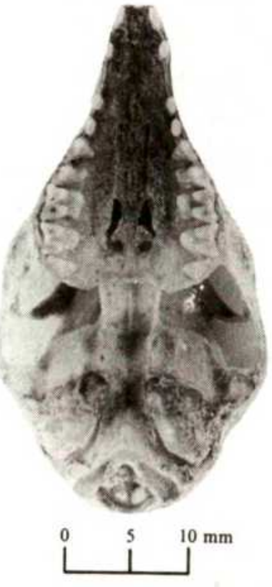

Fig. 2 *Tupaia* sp. Ventral view of the skull.

and P^3. All the teeth from P^3 to M^2 are three-rooted. P^3 and P^4 are bicuspid, the former with a distinct but poorly developed protocone and the latter with quite a prominent and well developed protocone. A complete and fully developed buccal cingulum is also present in P^4. M^1 and M^2 are triangular and dilambdodontic. Along the buccal margin of M^1 and M^2 are three prominent styles, parastyle, mesostyle, and metastyle. There is no hypocone or lingual cingulum on M^1 and M^2.

PUA I-3 differs from *Ptilocercus* in having a supraorbital foramen, well separated lacrimal and infraorbital foramena, a single-rooted canine, large P^3, mesostyles on the molars, no distinct cingulum and hypocones on the molars, and in the size of the incisors position of the orbit in relation to the teeth.

In the subfamily Tupaiinae it differs from *Anathana* which has the orbit (the anterior end) above P^4 rather than M^1. It can be differentiated from *Urogale* by the position of the orbit (above M^2) and the shorter face. It differs from *Dendrogale* in having a comparatively broader snout, single-rooted canine and a more transverse P^4. The specimen resembles *Tupaia minor* in size but differs in having a proportionately longer face, less posterior incisive foramen, distinct protocones on P^3 and more transverse P^4.

On the basis of the differences from various genera of Tupaiinae and the resemblance to Tupaia our specimen is clearly closer to *Tupaia*. But in view of morphological differences and the large age gap between our specimens and the living genus it has been assigned to a new genus and species *Palaeotupaia sivalicus*.

The specimen numbers YGSP 8089 and 8090 may also belong to this new taxon. But this can only be confirmed by further detailed studies of the material.

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The significance of beak marks on the wings of an aposematic, distasteful and polymorphic butterfly

D. A. S. Smith

Department of Biology, Eton College, Windsor, Berks, UK

Birds are the principal predators of adult butterflies[1] which they usually hunt on the wing. When successful, they often carry the prey to a perch and attempt to remove the wings with their beaks before eating. If the initial taste is repellant, the butterfly may be allowed to escape more or less uninjured except for a segment (beak mark) removed from its wing. There is evidence that beak-marked specimens are more common (in museum collections) in taxa known to be distasteful or toxic[2]. Based on field data from a population of *Danaus chrysippus* (L.) (Danaidae), a well known distasteful species[3], I have found that large individuals of both sexes are more frequently beak marked than small ones. Comparing two colour morphs (Fig. 1), the rarer one carried more beak marks.

The study was carried out on 235 hectares of grassland with scattered trees at Dar es Salaam, Tanzania. Butterflies were collected with a net, along regular routes, several times a week for 11 consecutive months. Each specimen was marked (to avoid double counting) and the sex, morph, forewing length and beak marks were recorded before release. Relative density was estimated as the number caught per hour.

Of 1,595 butterflies caught, 117 (7.3%) had beak marks. The distribution of beak marks was non-random in two respects. First, beak-marked butterflies were larger ($\bar{x}$ for forewing length = 38.6 mm) than unmarked specimens ($\bar{x} = 37.1$ mm), $d = 4.9$, $P < 0.001$. Second, of the two morphs studied (Fig. 1), *aegyptius* was more frequently beak-marked (12.7%) than *dorippus* (6.0%), $d = 4.1$, $P < 0.001$. Overall frequency of beak marks (y) was positively correlated with size of prey (x_2) ($r_{x_2y} = 0.614$, d.f. 9, $P < 0.05$) and frequency of *aegyptius* (x_3) ($r_{x_3y} = 0.76$, d.f. 9, $P < 0.01$) but not with density (x_1) (Table 1).

Several observers have claimed that birds tend to choose the largest prey available (ref. 4 and refs therein). Therefore, the higher frequency of beak marks on large butterflies might indicate that they are more subject to predation[5]. However, my data show that, although males are significantly larger ($\bar{x} = 38.007$ mm) than females ($\bar{x} = 36.020$ mm) ($d = 12.9$, $P < 0.001$), beak marks are equally frequent in both sexes ($X_1^2 = 0.4$, $P > 0.5$) and independently so in both morphs (for *dorippus*, $X_1^2 = 0.3$, $P > 0.5$; for *aegyptius*, $X_1^2 = 0.6$, $P > 0.3$). This

Table 1 Summary of the data relating to beak-marks on *D. chrysippus* at Dar es Salaam, 1974–5

Month	x_1 Capture rate (N h^{-1})	x_2 Mean forewing length (mm)	x_3 per cent* *aegyptius*	y per cent* beak marked	N
November '74	8.84	34.87	10.0	1.4	76
December	6.32	36.52	5.5	1.4	86
January '75	15.46	37.12	6.2	0.9	206
February	17.19	36.20	6.3	3.1	283
March	19.40	36.10	6.5	9.7	167
April	9.75	35.94	18.8	5.8	78
May	9.64	36.34	25.8	5.3	170
June	12.89	37.61	36.1	15.1	174
July	14.87	39.43	36.5	8.3	275
August	18.17	38.54	35.5	13.7	106
September	11.75	38.49	27.8	16.5	70

* arcsin transformed for statistical tests.

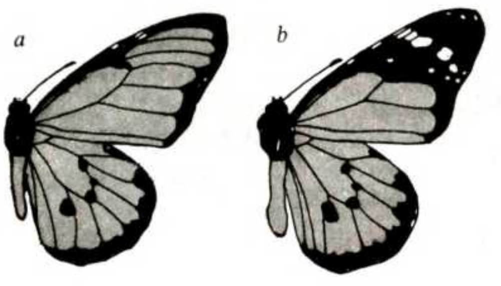

Fig. 1 *Danaus chrysippus* morphs *dorippus* (*a*) and *aegyptius* (*b*). Stippled areas are nut-brown to tawny orange, the remainder being black and white as shown.

unexpected result suggests that the same proportion of each sex is captured: moreover, females, though smaller and presumably weaker, escape predation after capture as readily as males.

The commonest food-plants (all Asclepiadaceae) available to *D. chrysippus* at Dar es Salaam[6] are *Calotropis gigantea*, which contains cardiac glycosides (cardenolides)[3] and *Tylophora stenoloba* which is non-toxic[7]. Butterflies (both sexes) reared on the former in the laboratory are significantly larger[6]. (Cardenolides are ingested by the caterpillar and stored in subsequent stages rendering them unpalatable to birds[3,8–10]. In *D. plexippus*, cardenolides are especially concentrated in the wings[11] where they are likely to be tasted by the bird before the body is fatally damaged.) Thus, the similar proportion of beak marks in both sexes of *D. chrysippus* probably results from the larger individuals of each sex containing more cardenolide, being more distasteful and, consequently, more often rejected unharmed. There is no evidence that the larger size of males serves to protect females.

As the morphs do not differ significantly in size in either sex, they probably use the same range of food-plants[6] and share a common palatibility spectrum. When reared on *C. gigantea* in the laboratory at Dar es Salaam, the two morphs contained similar amounts of cardenolide[3]. Therefore, the higher frequency of beak marking on both male ($X_1^2 = 10.9, P < 0.001$) and female ($X_1^2 = 4.1, P < 0.05$) *aegyptius* is unlikely to result from palatibility differences between the morphs. Moreover, within the range of my data, neither the density of the species as a whole nor the density or frequency of *aegyptius* itself had a significant effect on its beak-mark frequency.

The safety of a model is threatened if it has too many relatively edible mimics[12,13]. At Dar es Salaam, the commonest mimic of *D. chrysippus* is *Hypolimnas misippus* L. (Nymphalidae) which has two female morphs, *misippus* and *inaria*, mimicking *aegyptius* and *dorippus* respectively[14–15]. If *H. misippus* is relatively poorly protected, the *misippus* form, which is much more common than *inaria*[15], may constitute a threat to its *aegyptius* model by rendering the association as a whole relatively palatable. *H. misippus* females are rarely beak marked (3.2%, $n = 94$ in this study), suggesting they are usually consumed after capture. Furthermore, several common species of the well protected family Acraeidae[16] closely resemble *dorippus* and are, therefore, its müllerian co-mimics. *Acraea encedon* alone has forms mimicking both *D. chrysippus* morphs. Thus, *dorippus* is probably better protected and, consequently, relatively avoided and less beak marked by virtue both of its more common müllerian and rare batesian mimics.

A more detailed analysis and discussion of these results will be published elsewhere.

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Brassinolide, a plant growth-promoting steroid isolated from *Brassica napus* pollen

Michael D. Grove*, Gayland F. Spencer & William K. Rohwedder

Northern Regional Research Center, Science and Education Administration, US Department of Agriculture, Peoria, Illinois 61604

Nagabhushanam Mandava*, Joseph F. Worley, J. David Warthen Jr & George L. Steffens

Beltsville Agricultural Research Center, Science and Education Administration, US Department of Agriculture, Beltsville, Maryland 20705

Judith L. Flippen-Anderson

Laboratory for the Structure of Matter, Naval Research Laboratory, Washington, District of Columbia 20375

J. Carter Cook Jr

School of Chemical Sciences, University of Illinois, Urbana, Illinois 61801

Extracts of *Brassica napus* L. (rape) pollen (family, Cruciferae) have been reported to produce a novel growth-promoting effect when applied to young pinto bean plants[1,2]. Fractions that are active in the bean second-internode assay[3] give rise to both increased cell elongation and cell division[4]. The validity of these early reports has been questioned because biological activity was not correlated with any one pure compound[5]. Subsequently, we showed that isolation of the growth promoter was complicated by the presence of chromatographically similar, inactive components that were identified as 6-D-glucopyranosyl esters of long-chain fatty acids[6]. We now report the isolation, structure determination and biological activity of this new plant growth promoter, named (22*R*, 23*R*, 24*S*)-2α-3α,22,23-tetrahydroxy-24-methyl-6,7-s-5α-cholestano-6,7-lactone or brassinolide (Fig. 1).

A partially refined 2-propanol extract[7] of bee-collected rape pollen was subjected to successive chromatographic separations on silica gel (chloroform–methanol, 9:1 v/v; toluene–absolute ethanol, 9:1), on C_{18} Hi-flosil (methanol–water, 6:4), and by high-performance liquid chromatography on μ-Bondapak C_{18} (methanol–water, 65:35). The bean second-internode bioassay[3] was used to guide fractonation. Brassinolide (4 mg from 40 kg of pollen) was crystallised (MeOH) and has the following physical properties: $C_{28}H_{48}O_6$ [high-resolution field desorption mass spectrometry shows m/e 481.3489 $(M+1)^+$, calculated 481.3528]; melting point 274–275 °C; IR (KBr) 3,450, 1,690 cm^{-1}, (CH_2Cl_2) 1,720 cm^{-1}; ^{1}H-NMR (C_5D_5N) δ0.72 (s, 3H), 1.03 (d, 6H, $J = 6.3$ Hz), 1.04 (s, 3H), 1.10 (d, 3H, $J = 6.4$ Hz), 1.13 (d, 3H, $J = 6.5$ Hz); ^{13}C-NMR (CD_2Cl_2–CD_3OD, 9:1) δ10.3, 11.9, 12.1, 15.6, 20.8, 21.0 (CH_3), 68.3, 68.4, 71.0, 73.7, 74.8 (C–O), 177.6 (C=O).

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* To whom correspondence should be addressed.

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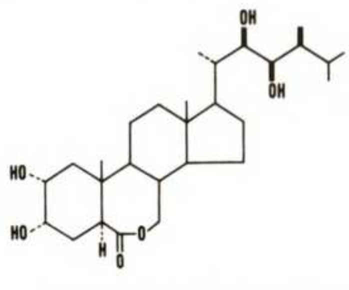

Fig. 1 Structure of brassinolide.

Field desorption mass spectrometry produced fragment ions at *m/e* 463 $(M+1-H_2O)^+$, 445 $(M+1-2H_2O)^+$, 409, 379, 361, 349, 131, 101 and 71. Chemical ionisation mass spectrometry (CI-MS) (isobutane reagent gas) showed ions at *m/e* 481, 463, 445, 427 $(M+1-3H_2O)^+$, 409, 379, 361, 349, 343, 321, 303 and 279. High-resolution electron impact mass spectrometry showed ions for $C_{22}H_{36}O_5$ at *m/e* 380.2561 (calculated 380.2562) and $C_{22}H_{33}O_4$ at *m/e* 361.2398 (calculated 361.2378). Acetylation of brassinolide produced a tetraacetate: *m/e* 649 $(M+1)^+$ by CI-MS. The mass spectral fragmentation pattern is consistent with cleavages along a dihydroxylated side chain and D-ring of a steroid (Fig. 2).

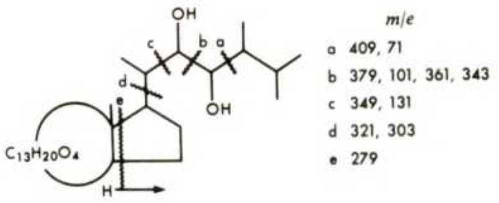

Fig. 2 Mass spectral fragmentation of brassinolide.

The structure of brassinolide was completely established by X-ray analysis of a single crystal which belonged to the monoclinic space group $P2_1$ with $a = 9.88(2)$ Å, $b = 7.63(2)$ Å, $c = 17.98(3)$ Å, and $\beta = 91.9(1)°$. There is one molecule of brassinolide and one molecule of water in the asymmetric unit. A partial structure was obtained by application of the symbolic addition procedure for non-centrosymmetric crystals[8]. The fragment was developed into the full structure by the tangent formula refinement and expansion method[9]. A perspective drawing of brassinolide less hydrogens (Fig. 3) was obtained using experimentally determined atomic positions at an intermediate stage of least-squares refinement. Although only the relative configuration was determined by the X-ray analysis, the enantiomer chosen for Fig. 3 depicts the normal absolute configuration found in natural steroids.

When tested in the bean second-internode bioassay, crystalline brassinolide produced an approximately 200% increase in elongation of the internode over that of an untreated control (Table 1). The increased cell division response was manifested by curvature, swelling and, most dramatically, by splitting of the second internode (Fig. 4) at concentrations of brassinolide as low as 10 ng per plant. Treatment with alkali destroys activity, which is regenerated on acidification.

Table 1 Bean second-internode activity of brassinolide

Brassinolide (ng per plant)	Percentage increase in elongation over untreated control	No. of split internodes
1,000	235	3 of 3
100	189	3 of 3
10	182	1 of 3
1	21	0 of 3

Bioassay was carried out as previously described[3,4]. Brassinolide was dissolved in dichloromethane-2-propanol before mixing with the fractionated lanolin carrier. Three plants were used for each concentration of brassinolide. An equal number of plants treated with lanolin carrier alone served as the control.

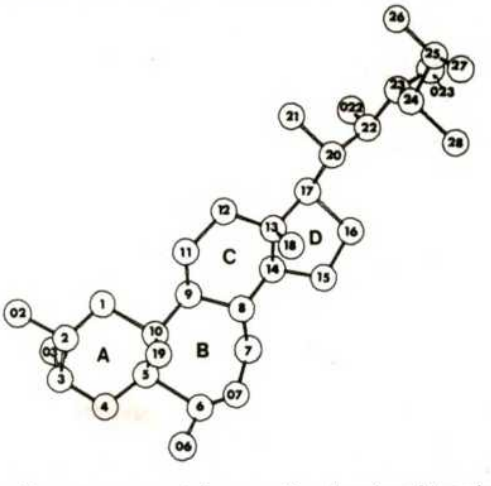

Fig. 3 Computer-generated perspective drawing of brassinolide. Hydrogens are omitted for clarity.

The brassinolide structure can be compared with ecdysone insect moulting hormones[10]. The 2,3-*cis*-glycol of brassinolide is α- rather than β-orientated; brassinolide has a *trans* A/B-ring fusion whereas ecdysones are *cis*-fused; ecdysones have a 7-ene-6-one grouping, whereas brassinolide has a B-ring lactone which is unprecedented in a natural steroid. Although the biosynthetic source of brassinolide is unknown, another C_{28} steroid, 24-methylenecholesterol, comprises 56% of the rape pollen sterols[11]. The 6,7-lactone functionality could originate from a 6-keto steroid precursor through a Baeyer–Villiger-type reaction. Oxidation of 3β-acetoxycholestan-6-one was reported to result in migration of either C-7 or C-5, with the brassinolide-like 6,7-lactone predominating[12].

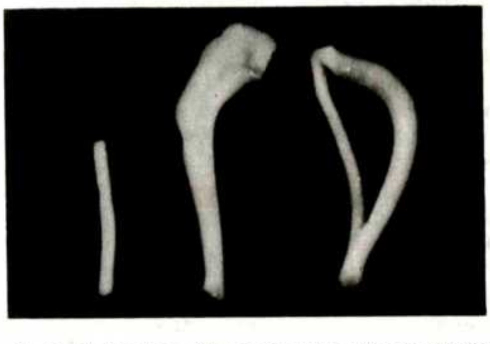

Fig. 4 Excised pinto bean (*Phaseolus vulgaris*) second internodes illustrating from left to right: untreated control; extremely swollen brassinolide treated; split brassinolide treated.

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Loss of immune competence with age may be due to a qualitative abnormality in lymphocyte membranes

Robin E. Callard* & Antony Basten

Immunology Unit, Department of Bacteriology, University of Sydney, NSW, 2006 Australia

Robert V. Blanden

John Curtin School of Medical Research, Australian National University, Canberra, 2600 Australia

The loss of immune function which occurs with age has until recently been attributed to depletion of immunocompetent cells[1–3]. However, studies from our laboratory[4–6] have indicated that the age-related decline in immune responsiveness is better explained by a qualitative rather than a quantitative defect at the level of both T cells and B cells. This conclusion was derived from experiments in which immune function was assessed in relation to the number of responding cells. Thus, lymphoid tissues from old and young mice were found to contain comparable numbers of T cells and B cells despite a 10-fold or greater difference in their antibody-forming potential and mitogenic activity. Furthermore, selective depletion of reactive subpopulations was excluded by the demonstration of similar proportions of mitogen and antigen-binding cells in lymphoid tissue from old and young mice. If the number of cells is normal in old animals, but their function is impaired, it is possible that the decline in immune competence with age may be due to a metabolic or structural abnormality, perhaps affecting the cell membrane and preventing the cell from responding normally to exogenous signals. We have therefore investigated whether any difference could be detected in membrane-associated antigens of lymphoid cells from old and young mice of the same strain. We report here that new or altered antigenic determinants seem to be expressed on the surface of lymphocytes from old mice. These might interfere with the normal immunological activities of such cells, and so perhaps explain their reduced immune competence.

Three separate techniques were used in our studies. First, spleen cells from six young (3–4 months), C57BL/6J mice were cultured with spleen cells from six syngeneic old (24–30 months) mice of the same sex in one-way mixed lymphocyte reactions (MLRs). Cells were prepared separately from each mouse and cultured in all possible responder-versus-stimulator combinations. As shown in Table 1, except for three young→old combinations, spleen cells from each of the six young donors responded to spleen cells from each of the six old mice. Conversely, cells from each old mouse responded to stimulator cells from each young donor. At no time was any response obtained in young→young and old→old combinations. These findings suggest that cells from old mice possess cell-surface determinants which can stimulate proliferation of young cells and vice versa. This observation was confirmed by the demonstration of a similar young→old MLR with spleen cells from another strain, CBA/Ca/T$_6$ (Table 2). Furthermore, the addition of polyethylene glycol, which potentiates weak MLRs[7], significantly enhanced the young→old syngeneic MLR of cells from both C57BL/6J and CBA/Ca mice (Table 2). This supports the notion that the response obtained in young→old combinations of syngeneic cells represents a true MLR elicited by weak antigenic differences.

The second approach used for the detection of differences in membrane-associated antigens involved raising an antiserum to lymphoid cells from old mice in young syngeneic recipients (young anti-old). This young anti-old serum bound to 7–8% of spleen and lymph node cells from old mice but to only 1–2% of lymphoid cells from young mice (Table 3). Neither young anti-young control serum nor normal sera collected from both groups of recipients before immunisation bound to more than 2% of cells from old or young mice; this is no greater than the number of cells which label with protein A alone. It may be due to the presence of protein A antigen-binding cells or binding of protein A to membrane-associated IgG which is normally present on 1–2% of normal mouse spleen cells[8]. The ability of young anti-old serum to recognise determinants on old lymphoid cells was confirmed by complement-dependent cytotoxicity by the method of Boyse *et al.*[9] (Table 3).

Table 1 Syngeneic one-way mixed lymphocyte reaction between spleen cells from old and young mice

Source of responder spleen cells	Source of stimulator spleen cells: Young mice 1	2	3	4	5	6	Old mice 1	2	3	4	5	6
Young mice												
1	—	0	0	0	0	0	1	2	5	1	0	4
2	0	—	0	0	0	0	1	3	4	1	3	3
3	<1	0	—	0	0	0	2	2	5	2	0	3
4	0	0	0	—	0	0	1	3	3	1	0	4
5	0	0	0	0	—	0	2	3	3	2	3	2
6	<1	<1	0	0	0	—	3	4	2	3	2	6
Old mice												
1	4	2	5	1	5	4	—	0	0	0	0	0
2	4	3	6	6	7	5	0	—	0	0	0	0
3	3	4	3	4	1	1	0	0	—	0	0	0
4	6	4	4	6	5	3	0	0	0	—	0	0
5	4	3	3	2	1	3	0	0	0	0	—	0
6	4	4	4	4	5	4	0	0	0	0	0	—

Spleen cells were prepared separately from six young and six old C57BL/6J mice of the same sex and cultured in all possible combinations in a one-way MLR. Quadruplicate cultures were carried out in Linbro microtitre trays (Flow Laboratories). Each well contained 5×10^5 stimulator cells and 5×10^5 responder cells in 0.2 ml medium RPMI 1640 (GIBCO) containing 2.5% fetal calf serum (FCS) (Flow Laboratories), 5×10^{-5} M 2-mercaptoethanol, 20 mM HEPES (Flow Laboratories), 2 g l^{-1} NaHCO$_3$, 60 μg ml^{-1} penicillin and 100 μg ml^{-1} streptomycin. Cell division was prevented in stimulator cells by exposure to 1,500 rads irradiation from a ^{60}Co source. Cultures were incubated at 37 °C in an humidified atmosphere of 5% CO$_2$ in air for 48 h, pulsed with 0.5 μCi of ^{3}H-thymidine and collected 24 h later with a Skatron automatic harvester. Incorporation of ^{3}H-thymidine was determined by counting in 0.5% diphenyloxazole/toluene scintillant using an LKB Wallac 81000 liquid scintillation counter. Counts were corrected for efficiency and quenching and expressed as d.p.m. The results are expressed in significant ($P<0.05$) d.p.m.$\times10^{-3}$ above background (–). Background counts were obtained from cultures of spleen cells with irradiated cells from the same animal and were generally less than 5,000 d.p.m. Cultures in which d.p.m. were not significantly above background are expressed as 0. It can be seen that in all but three combinations, cells from each young mouse (young 1–6) responded significantly above background to cells from each of the six old donors. Similarly, cells from each of the six old donors (old 1–6) responded to young but not old cells.

The third group of experiments was designed to test whether the new determinants expressed on old cells might involve antigens of the major histocompatibility complex (MHC), in view of their known importance in modulating immune responsiveness. Reciprocal skin grafts were therefore exchanged between old and young C57BL/6J mice of the same sex. These, however, remained intact for longer than 60 d, suggesting that any modification of cell-surface components with age did not result in significant alteration to either major or minor histocompatibility antigens involved in graft rejection. Possible changes in H-2K and H-2D antigens were further tested for by using an 'H-2-restricted' cytotoxic T-cell response to viral infection[10]. Cytotoxic T cells specific for H-2K or H-2D plus viral antigens were generated in young C57BL/6J mice. These cells lysed virus-infected peritoneal macrophage target cells from old and young mice with similar efficiency (Table 4). As this assay is very sensitive to qualitative changes in H-2K or H-2D antigens caused by mutations[11,12], the negative results obtained

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* Present address: ICRF Human Tumour Immunology Group, University College Hospital Medical School, University Street, London WC1, UK.

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Table 2 Enhancement of syngeneic young→old MLR with polyethylene glycol

Source of spleen cells used in syngeneic MLR	Polyethylene glycol added No	Yes
C57BL/6		
Young→old	2,700±484	8,100±1,750
CBA		
Young→old	9,900±1,300	14,370±3,900

Syngeneic young→old MLRs using spleen cells from two strains of mice were carried out with and without 5% polyethylene glycol, which is known to enhance weak MLRs. Results are expressed as d.p.m. above background counts obtained from young→young MLR cultures. Young mice were aged 3–6 months and old mice 24–30 months.

suggest that an age-related change in H-2K or H-2D antigens is unlikely, but do not exclude alterations in I-region antigens which could explain the stimulation seen in the MLR experiments (Tables 1, 2).

The above findings suggest that new or altered antigenic determinants are expressed on the surface of lymphoid cells from old mice. Antigenic differences between populations of syngeneic lymphocytes have also been reported previously in other systems. For example, cell-mediated cytotoxicity has been obtained in female mice against male-specific (H–Y) antigens[13], although such an explanation has been excluded in the present study by the exclusive use of mice of one sex. In addition, mixed lymphocyte reactivity has been demonstrated with combinations of neonatal thymocytes and syngeneic adult spleen cells[14], which is consistent with the appearance of new antigens during cellular differentiation. More recently, Gozes *et al.*[15] have demonstrated a syngeneic graft-versus-host (GvH) response induced in popliteal lymph nodes of young C57BL/6 mice by footpad injection of syngeneic spleen cells from old donors. This finding, as well as the young→old syngeneic responses reported here, could represent an autoimmune response to 'self' antigens or a response to new antigenic determinants on old lymphoid cells. Such new antigenic determinants could have arisen in various ways; for example, by changes in the cholesterol/phospholipid ratio in the plasmalemma (of the type reported by Rivnay *et al.*[16]) which alters membrane viscosity, disturbs normal lymphocyte function and may expose hidden antigenic determinants, or by insertion, into the lymphoid cell membrane, of altered proteins arising by somatic mutation or from frequent errors in protein synthesis, both of which have been postulated as causes of cellular ageing[17,18].

Table 3 Specific binding and complement-mediated cytotoxicity of young anti-old serum against lymphoid cells from old mice but not young mice

		% Of lymphocytes labelled with:		
Donor mice	Source of cells	Young anti-old serum	Young anti-young serum	Normal mouse serum
Old	Spleen	7.5±1.2*	2	2
Old	Lymph node	6.8±2.0	2	2
Young	Spleen	2	2	2
Young	Lymph node	2	2	2
		% Of lymphocytes killed with:		
		Young anti-old serum	Young anti-young serum	Normal mouse serum
Old	Spleen	14.2±1.2	7.0±0.6	6.0±0.6
Young	Spleen	7.0±1.3	6.4±1.1	6.0±0.8

Antisera specific for old lymphoid cells were raised in syngeneic young recipients of the same sex by six injections of 10^7 pooled spleen and lymph node cells from old C57BL/6J donors given intraperitoneally at weekly intervals. The first, third and fifth injections were given as an emulsion in Freund's complete adjuvant, and viable cells were used on the remaining occasions. A control serum (young anti-young) was prepared in the same way except that young spleen and lymph node cells were substituted for old cells in the immunisation regime. Both antisera were collected 7 d after the final injection. All sera were tested on lymphoid cells from syngeneic mice of the same sex. Specific binding of anti-lymphocyte antibodies was determined autoradiographically, using ^{125}I-labelled protein A, which binds to the Fc portion of immunoglobulin[23]. Briefly, 5×10^6 spleen or lymph node cells were incubated with 20 µl of antiserum in 100 µl of medium 199 containing 0.5% bovine serum albumin (Commonwealth Serum Laboratories) and 15 mM sodium azide for 30 min on ice. After two washes, the cells were incubated with 0.5 µg of ^{125}I-protein A (protein A obtained from Pharmacia and iodinated by the method of Greenwood *et al.*[23] to a specific activity of 10 µCi per µg) in 0.4 ml medium. Unbound label was removed by centrifugation and the cells smeared on gelatin-coated slides. Slides were dipped in Kodak NTB2 emulsion and exposed for 10–21 d at 4 °C and then developed in Kodak D19. The cells were stained with Giemsa and the proportion of labelled small mononuclear cells determined from counts of 1,000 cells per slide. Complement-dependent cytotoxicity was carried out according to the method of Boyse *et al.*[9].

* Mean value ± s.e.m.

Table 4 Lysis of ectromelia virus-infected peritoneal macrophages from old or young C57BL/6J mice by immune spleen cells from young syngeneic donors

	^{51}Cr Release		
Source of† target cells	Water lysis	Spontaneous lysis	Lysis caused by immune spleen cells
Virus-infected macrophages from old mice	93.5±0.5	34.5±2.0	72.4±2.1* 51.7±3.7*
Control (uninfected) macrophages from old mice	90.1±1.1	22.8±1.2	21.9±0.1 27.0±0.8
Virus-infected macrophages from young mice	92.8±1.4	33.6±2.3	79.3±0.8 46.4±2.2
Control (uninfected) macrophages from young mice	87.4±2.2	24.0±0.8	24.4±1.5 24.7±1.0

* Results obtained from two individual immune mice given 10^4 plaque-forming levels of ectromelia virus intravenously 5 d previously. The killer : target ratio was 20 : 1 and the assay was run for 16 h.

† Macrophages were infected with 40 plaque-forming units of virus for 1 h at 37 °C and left for 3 h before addition of immune spleen cells as described in detail elsewhere[11].

The nature of the new antigenic determinants detected on lymphoid cells from old mice is unknown but the current experiments do shed light on some of the possibilities. The lack of skin graft rejection between old and young mice and the failure to detect differences in cytotoxic T-cell activity (Table 4) argue against alterations in K or D antigens of the MHC. However, neither experiment excludes changes in Ia or M locus antigens which may not give rise to graft rejection. Consistent with modifications in I-region determinants is the finding of mixed lymphocyte reactivity between old and young cells from two different strains of mice (Tables 1, 2). Moreover, further evidence suggests altered expression of Ia antigens in old mice. For example, macrophages from old mice are abnormal in their ability to function as stimulator cells in an allogeneic MLR[19] and an increase in I-J-bearing T cells corresponding to the presence of T-cell suppressors has been detected in old mice (R.E.C. *et al.*, in preparation). An alteration in I-region products with age could explain the decline in lymphocyte function with age, as Ia antigens have a key role in cellular interactions of the immune response. Also, modified Ia antigens may contribute to the high incidence of neoplasia and autoimmunity in the aged. This is supported by the finding of Gleichmann *et al.*[20] of an increased incidence of lymphomas and autoimmunity in chronic GvH disease involving I-region differences only. In addition, the rise in I-J-bearing suppressor cells may further exacerbate the development of tumours[21]. These membrane changes may also explain the high incidence of autoimmune disease in the aged: insertion of a new, non-self, antigen onto the lymphocyte cell surface could allow T-cell help to be expressed for antibody responses to other, self surface antigens. The alternative explanation of autoimmune reactivity to self antigens resulting from a loss of T-cell suppressor function seems less likely because an increase rather than a decrease in T-cell suppressor activity occurs in old mice[6,22]. Whatever the exact nature of the new antigen determinant on old lymphoid cells, it is clear that it may profoundly affect normal immune functions. Furthermore, accumulation of qualitative abnormalities of this kind in other somatic cells may account for ageing in a wide variety of higher organisms.

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Immunity to human haematopoietic stem cells

Robert Peter Gale & Caprice Rutkosky Moran

UCLA Bone Marrow Transplant Team, UCLA School of Medicine, Center for the Health Sciences, Los Angeles, California 90024

Graft rejection is an important problem in human bone marrow transplantation, with a 25–60% incidence in grafts between HLA-identical siblings[1–6]. In mice, two models of rejection of bone marrow grafts have been described: T-lymphocyte-mediated immunity and hybrid resistance (for reviews see refs 7, 8). The first model involves the recognition of foreign histocompatibility (H-2) antigens by selected subsets of T lymphocytes. These antigen-reactive cells undergo clonal expansion and differentiate into cytotoxic effector cells which mediate graft rejection. In the hybrid resistance model, mononuclear cells, probably identical to natural killer (NK) cells, react against haematopoietic histocompatibility antigens on parental marrow cells[9]. Cells involved in hybrid resistance exhibit 'spontaneous' cytotoxicity to disparate haematopoietic cells and, in contrast to T-lymphocyte-mediated immunity, do not require a sensitisation phase before differentiating into cytotoxic effector cells. The mechanism of graft rejection following bone marrow transplantation in man is unknown. In transplants between HLA-identical siblings the target antigens of graft rejection are not HLA or antigens closely linked to the major histocompatibility complex (MHC). To study the problem of graft rejection in man we developed and describe here an *in vitro* model to detect immunity to haematopoietic stem cells committed to granulocyte–macrophage differentiation (CFU-C; colony-forming unit culture). We found that human peripheral blood and bone marrow but not thymus contain a population of cells which are cytotoxic to haematopoietic stem cells following *in vitro* sensitisation. The effector cell population is not a conventional T or B lymphocyte, monocyte–macrophage or NK cell.

The model system is shown in Fig. 1. Peripheral blood mononuclear cells (PBMC) were co-cultivated with irradiated bone marrow cells for 5–7 days. Sensitised PBMC were recovered and incubated with fresh, unirradiated bone marrow cells for an additional 16 h after which the PBMC–bone marrow mixture was suspended in semi-solid agar and plated into Petri dishes over an underlayer containing human colony-stimulating activity (CSA). Granulocyte–macrophage colonies (CFU-C) were counted 7–14 days later using an inverted microscope[10,11].

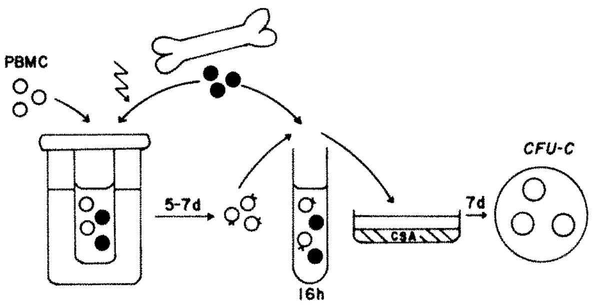

Fig. 1 Experimental model of immunity to granulocyte–macrophage progenitors (CFU-C). Peripheral blood mononuclear cells (PBMC), obtained by Ficoll–Hypaque density centrifugation of venous blood, were resuspended in McCoys 5A medium, 20% fetal calf serum (FCS) and 1% penicillin–streptomycin (PS). Bone marrow from normal donors was obtained by needle aspiration from the posterior iliac crest into syringes containing preservative-free heparin. A leukocyte-rich bone marrow cell population was prepared by centrifugation in Wintrobe tubes at 1,600*g* for 10 min. 10^6 PBMC (0.5 ml) were co-cultivated with an equal number of irradiated bone marrow cells (1,500 rads, Gammacell-220 ^{60}Co-source; rate, 3,200 rad min^{-1}) in Marbrook flasks for 5–7 days at 37 °C in a 7.5% CO_2 atmosphere. Sensitised PBMC were recovered, washed and resuspended in McCoys 5A, 20% FCS and 1% PS. Sensitised PBMC (10^6, 1 ml) were cultured with an equal number of bone marrow cells in 16 × 125 mm plastic tubes for 16–24 h at 37 °C, 7.5% CO_2 atmosphere. Cells (2×10^5) were then diluted in 0.3% agar in McCoys 5A with 15% FCS and plated over white blood cell underlayers in 0.5% agar in Petri dishes which were incubated for 10 d at 37 °C. Colonies of ⩾40 cells were counted using an inverted microscope[10,11].

Data were expressed as the $\bar{X} \pm$ s.e.m. of triplicate cultures and were analysed by paired t-test or corrected χ^2 analysis as indicated. Representative data of five experiments are shown in Fig. 2. Normal human marrow has a cloning efficiency of 104 ± 9 CFU-C and normal PBMC less than 10 CFU–C per 2×10^5 cells plated in these conditions. The addition of PBMC co-cultured with autologous bone marrow to fresh, unirradiated bone marrow from unrelated donors consistently increased the number of CFU-C, probably related to CSA release. In contrast, PBMC sensitised to allogeneic bone marrow consistently inhibited the CFU-C in fresh bone marrow from donors identical or unrelated to the donor of the sensitising cells (Fig. 2). No CFU-C inhibition was observed when bone marrow from the PBMC donor was used as a target or in cultures between genetically identical twins. These data exclude a nonspecific effect of inhibition of CFU-C by *in vitro* sensitisation

The interaction between sensitised PBMC and target CFU-C is time and dose dependent and requires cell–cell contact (Fig. 3). Twenty per cent inhibition of CFU-C was observed after 2–4 h and 80% after 16 h of secondary culture. A 2 : 1 ratio of PBMC to bone marrow usually resulted in a 70–80% inhibition of CFU–C. CFU-C inhibition was not observed when sensitised PBMC were separated from target bone marrow cells by either a Nucleopore or Millipore membrane (control 104 ± 6; co-culture 22 ± 3; separated culture 97 ± 7).

Cell-mediated inhibition of CFU-C was relatively unaffected by depletion of T lymphocytes by anti-thymocyte globulin

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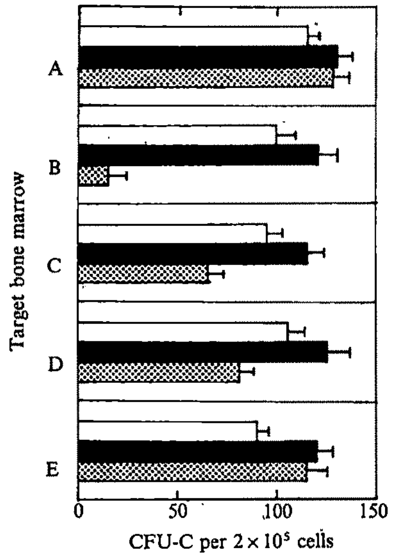

Fig. 2 CFU inhibition by sensitised effector cells. PBMC from individual A were sensitised to autologous bone marrow cells (black bars) or to bone marrow from an unrelated individual B (stippled bars). The sensitised cells were then co-cultured with fresh bone marrow cells and CFU-C determined. Target bone marrow from A and B and from three unrelated individuals (C, D, E) were also tested (ordinate). CFU-C content of the bone marrow from each donor without addition of sensitised effector cells is indicated by the clear bars.

Table 1 Effects of mononuclear cell subpopulation on CFU-C

	Mononuclear cell sub-population			
	T	B	M	NK
Control	86±4	112±7	96±7	75±4
Sensitised PBL	30±2	32±3	20±3	17±2
Depleted	32±5	26±3	17±5	33±6
Enriched	93±6	102±5	120±10	102±7

The CFU-C assay was carried out as described in Fig. 1 legend. PBMC from HLA-mismatched individuals were co-cultivated and tested for inhibition of bone marrow cells from the same donor as the sensitising PBMC. Results are expressed as follows: control, autologous PBL co-cultures with target bone marrow cells ($A \times A_x \rightarrow B$); sensitised, allogeneic PBMC co-cultures with target BM ($A \times B_x \rightarrow B$). HLA-identical siblings were used in the experiment involving T lymphocytes. In other experiments HLA-mismatched, unrelated donors were used. Sensitised PBMC were fractionated using techniques described in Fig. 4 legend. Subpopulations were depleted or enriched for T lymphocytes (E-rosettes), B lymphocytes (nylon-wool column) or macrophages (adherence). NK cells were isolated by fractionation on an immune complex monolayer as described elsewhere[22]. Results are expressed as CFU-C per 2×10^5 cells plated.

(ATG), or removal of sheep erythrocyte rosette-forming cells (E-RFC); by removal of B lymphocytes by nylon wool columns or with anti-B-lymphocyte antisera; or by removal of monocyte–macrophages by phagocytosis of carbonyl ion or adherence to plastic surfaces (Fig. 4). Concentrations of silica and carrageenan that completely inhibited NK activity also had no effect on PBMC-mediated CFU-C inhibition. Furthermore, purified populations of T and B lymphocytes, monocyte–macrophages and NK cells showed no inhibition of CFU-C in identical conditions (Table 1). Bone marrow-derived mononuclear cells but not thymocytes could also serve as a source of effector cells in this assay (data not shown). These data indicate that the effector cell is probably not a conventional T or B lymphocyte, monocyte–macrophage or NK cell.

The target antigen(s) on CFU-C recognised by sensitised PBMC is unknown. To evaluate the role of HLA antigens, we studied five pairs of HLA-identical siblings. Figure 5 shows representative data. CFU-C inhibition was reproducibly demonstrated in four of the five pairs of siblings, indicating that HLA antigens are not the primary target of CFU-C inhibition. In most instances, the degree of CFU-C inhibitions between HLA-identical siblings was not as great as that observed between HLA-mismatched combinations. Recently, B-lymphocyte-associated antigens (HLA-DR or Ia-like) have been demonstrated on CFU-C[12,13]. The possibility that this antigen was the target of CFU-C inhibition was investigated by experiments in which an antiserum to human B-lymphocyte antisera failed to block CFU-C inhibition by sensitised PBMC[14] (control 114±6; sensitised culture 27±7; sensitised culture with anti-Ia, 21±3). The CFU-C inhibition observed between HLA-identical siblings is further indirect evidence that B-lymphocyte-associated antigens are not important in this

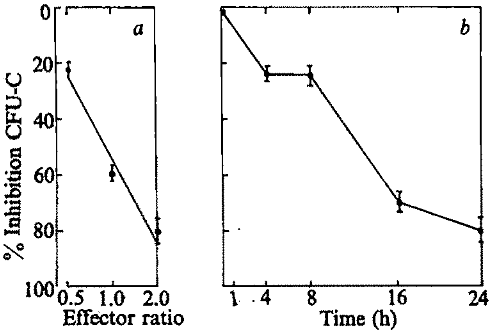

Fig. 3 Kinetics of CFU-C inhibition. Sensitised PBMC recovered from primary cultures were recultured with fresh bone marrow cells at various ratios (*a*) and for various lengths of time (*b*). The secondary cultures were then terminated and CFU-C assayed as indicated (Fig. 1).

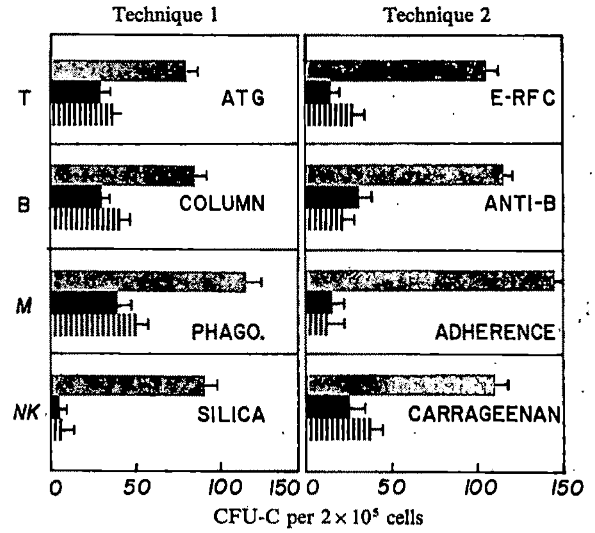

Fig. 4 Effects of cell fractionation on CFU-C inhibition in control cultures (stippled bars), sensitised cultures (black bars) and sensitised cultures depleted of selected mononuclear cell populations (striped bars) by the method indicated. Sensitised PBMC were fractionated using the procedures indicated. After each procedure the cells were washed, reconstituted to their original concentration and tested for CFU-C inhibition. Unfractionated sensitised PBMC served as a control. T-lymphocyte-depleted fractions were obtained by incubation of 10^7 PBMC (1.0 ml) with 0.25 ml equine anti-human thymocyte globulin (ATG; Upjohn–17912; $1:10^3$ dilution) and rabbit complement or by rosette formation with AET (2-aminoethylisothiouronium bromide hydrobromide) treated sheep erythrocytes (E-RFC) and centrifugation over a Ficoll–Hypaque density gradient[17]. T-depleted PBMC contained ≤5% E-rosette forming cells. B lymphotytes were depleted by adherence to nylon wool columns or by incubation with a rabbit anti-human B-lymphocyte antiserum and complement[18,19]. B-lymphocyte-depleted fractions contained ≤2% surface membrane Ig-positive ($SMIg^+$) cells. Monocyte–macrophage-depleted (M) fractions were obtained by incubation with carbonyl iron (lymphocyte separating reagent, Technicon) and passage through a laboratory magnet or by adherence to polystyrene surfces. Macrophage-depleted fractions contained ≤5% nonspecific esterase staining cells. NK cells were inactivated by incubation with silica or carrageenen. PBMC were incubated for 30 min with silica, 0.1–1.5 mg ml^{-1} (Sigma; particle size <0.4 μm). The silica was sonicated and used uncoated or after incubation with 10% FCS. PBMC were also incubated with carrageenen, 0.15 mg ml^{-1} (Hathaway Allied Products) for 60 min. Treated fractions were tested for NK activity against sensitive (K-562, MOLT-4) and resistant (RAJI) cell lines. In each instance NK activity was absent[20,21]. HLA-identical siblings were used in the experiments involving T lymphocytes. In other experiments HLA-mismatched, unrelated donors were used.

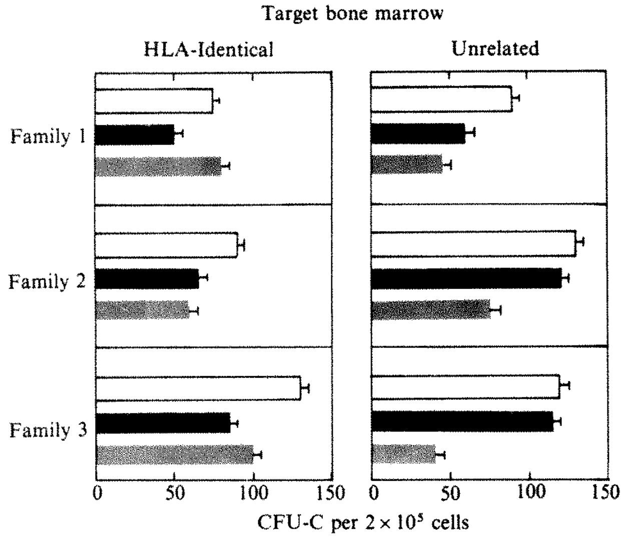

Fig. 5 CFU-C inhibition between HLA identical siblings. In three families with two HLA-identical siblings, PBMC from one sibling were sensitised against bone marrow cells from either the HLA-identical sibling (black bars) or an unrelated individual (stippled bars). Their sensitised PBMC were then tested for CFU-C inhibition by co-culture with fresh bone marrow from either the sibling (left panel) or the unrelated individual (right panel). The values indicated by the clear bars represents the number of CFU-C present when target bone marrow was cultured with PBMC sensitised against autologous bone marrow cells in primary culture.

system, as these antigens are closely linked or identical to the HLA-D locus shared by these siblings[15].

We have thus demonstrated that PBMC sensitised against allogeneic but not autologous bone marrow contain a population of cells which inhibit haematopoietic stem cells committed to granulocyte–macrophage differentiation (CFU-C). The effector cell is not a conventional T or B lymphocyte, monocyte-macrophage or NK cell. The target antigen(s) on the CFU-C is unknown but is not HLA or a B-lymphocyte-associated (HLA-DR or Ia-like) antigen. This system should prove useful for studying immunity to non-HLA antigens and as a model for bone marrow graft rejection in man.

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H-2 restriction of contact inhibition of epithelial cells

A. S. G. Curtis & Paul Rooney

Department of Cell Biology, University of Glasgow, Glasgow, UK

Medawar has suggested[1] that the major histocompatibility gene complex (MHC) might be involved in the contact inhibition of movement[2] shown by fibroblasts and epithelia. Contact inhibition of movement[3] is that reaction of cells which stops the movement of one cell over another and thus leads to monolayering and other features of morphology typical of cells in culture and *in vivo*[4]. By confronting epithelial outgrowths we have compared contact inhibition between syngeneic cells with that between allogeneic cells. Contact inhibition was more marked between allogeneic combinations than syngeneic combinations, when the genetic mismatch lay in certain parts of the MHC complex.

A number of cell interaction gene loci (CI loci) have been recognised in lymphocyte–lymphocyte and lymphocyte–macrophage interactions[5], controlling such reactions as T–B lymphocyte cooperation in antibody responses, and the T–T lymphocyte reactions in the genesis of cytotoxic lymphocytes (CTL)[6], as well as the reaction of the CTL with their target cell (which may be fibroblasts). These CI loci map in the MHC and, in mice, afford examples of H-2 restriction. If the CI loci actually control the interaction part of the process, rather than the response to the interaction, a question that has not been answered, we would already possess clear evidence that some cell interaction phenomena (rather than a consequent response) are under MHC control. There is evidence to show that cell adhesion of mouse fibroblasts is under H-2 control[7], that the adhesion of mouse lymphoid cells is under the control of one or more of the loci K, IA, IB, D or L (though minor effects of others could not be excluded)[8], that the adhesion of mouse T lymphocytes is controlled by loci D and/or L and perhaps IB, IC and IJ (ref. 9), and that mouse B lymphocyte adhesion is controlled by loci K and perhaps IB and IC (ref. 10). In refs 9 and 10, clear evidence was presented that allogeneic combinations of cells, mismatched at these loci, led to a diminution of adhesion, effected by soluble glycoproteins secreted by the cells.

Epithelia, which would be expected to show marked contact inhibition of movement[11], were grown as outgrowths of explants of adult mouse kidney tubule and the extent of contact inhibition measured (comparing the number of nuclear overlaps with the number expected on random grounds) in the area of confrontation between syngeneic or allogeneic outgrowths. The congeneic strains B10, B10.A, B10.A(2R), B10.A(4R), B10.AKM, B10.G, B10.BR and B10.HTT were used, and also strain ATL. The results are shown in Table 1.

The results show that although there is a low overlap index (marked contact inhibition of movement) in the syngeneic controls, the allogeneic combinations show a very low overlap index, significantly below that of the controls, when certain loci are mismatched between the two strain types in the culture. In other words, there is an H-2 restriction affecting the extent of contact inhibition. If the cells involved are mismatched at K or/and D, contact inhibition is markedly increased.

It might be expected that in a culture dish containing equal numbers of explants from two strain types, 50% of the confronted outgrowths would be syngeneic and 50% allogeneic, and thus, that a population of overlap indices with two (possibly three) different means corresponding to two syngeneic and one allogeneic groups. Yet, all confronted outgrowths in such cultures show a very low overlap index (see Table 1.). A simple

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Table 1 Contact inhibition of movement of mouse kidney tubule epithelium in allogeneic and syngeneic confrontations

Strain	Overlap index*	Haplotype									H-2 mismatch	*t*-test	
Multi-explant cultures		K	IA	IB	IJ	IE	IC	S	G	D			
B10.G	0.27 (0.12)	*q*	*q*	*q*	*q*	*q*	*q*	*q*	*q*	*q*			
B10.BR	0.31 (0.24)	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*k*		= 6.01	
B10.G + B10.BR	0.11 (0.07)										All loci		= 4.55
B10.G	0.28 (0.12)	*q*	*q*	*q*	*q*	*q*	*q*	*q*	*q*	*q*			
B10.AKM	0.23 (0.13)	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*q*		= 7.47	
B10.G + B10.AKM	0.08 (0.06)										All but D		= 5.48
B10.BR	0.30 (0.12)	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*k*			
B10.AKM	0.23 (0.13)	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*q*		= 4.69	
B10.BR + B10.AKM	0.10 (0.06)										D only		= 5.48
B10.A(2R)	0.31 (0.06)	*k*	*k*	*k*	*k*	*k*	*d*	*d*	*d*	*b*			
B10.A(4R)	0.34 (0.11)	*k*	*k*	*b*	*b*	*b*	*b*	*b*	*b*	*b*		= 0.72	
B10.A(2R) + B10.A(4R)	0.34 (0.19)										IB, IJ, IE, IC, S & G		= 0.02
B10.HTT	0.29 (0.09)	*s*	*s*	*s*	*s*	*k*	*k*	*k*	*k*	*d*			
ATL	0.36 (0.12)	*s*	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*d*		= 0.10	
B10.HTT + ATL	0.30 (0.12)										IA, IB		= 1.97
B10	0.26 (0.09)	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*			
B10.A(4R)	0.27 (0.14)	*k*	*k*	*b*	*b*	*b*	*b*	*b*	*b*	*b*		= 7.15	
B10 + B10.A(4R)	0.10 (0.08)										K, IA		= 5.82
Microwell cultures 2 explants													
B10.BR	0.31 (0.15)												
B10.AKM	0.27 (0.14)												
B10.BR + B10.AKM	0.13 (0.07)										D only		

Data are from epithelial cultures grown as outgrowths from explants for 3 days in Hams F10 (modified with 0.02 M HEPES + 0.004 M $NaHCO_3$ *p*H7.4) and 10% fetal calf serum. Contact inhibition was measured as nuclear overlap index on areas of $7.08 \times 10^{-8} m^2$, 30 areas per culture type. $P = 0.01$ for $t = 2.66$, 58 d.f.

* Mean; s.d. in parentheses.

explanation of this effect would be that cells produce a soluble diffusible factor that affects the contact inhibition of cells allogeneic to the producer cells. Similar substances affecting cell adhesion have been described and characterised as glycoproteins from T and from B lymphocytes and have been shown to reduce the adhesion of allogeneic lymphocytes, if they are mismatched at H-2 D or L (T cells) or H-2 K (B cells)[9,10]. There is some evidence for Ia antigen involvement in adhesion[9,10]. It is likely that contact inhibition of movement is at least partially controlled by adhesion[3].

It is not yet clear how these soluble factors are received by the target cells. Interestingly, Snell[12], reviewing work on MHC receptors in T cells, points out that the I region may contain pairs of closely linked genes determining interacting cell surface structures. If any locus is involved in adhesion, the presence of allogeneic cells (or their factors) may lead to competitive inhibition of such sites and thus diminution of adhesion.

Bodmer[13] suggested that the true role of the MHC systems might be the control of cell–cell recognition. Normally, this recognition is, of course, predominantly a syngeneic phenomenon. The results we describe suggest that contact inhibition of movement, long believed to be involved in organogenesis, is under MHC control. Extreme contact inhibition seen in certain allogeneic combinations would prevent the interdigitation of cells required for the normal formation of tissues such as epithelia, and might, by itself, prevent proper fusion of allogeneic grafts. The problem yet to be solved is how syngeneic tissues within one organism differ sufficiently to separate and position themselves differently: one suggestion is that the display of MHC antigens varies between different tissues in such a way that pairs of cell types, one lacking and the other showing the expression of a certain locus, will interact in a different manner from those sharing its expression.

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Mechanical activation and voltage-dependent charge movement in stretched muscle fibres

Chiu Shuen Hui* & W. F. Gilly†

Department of Physiology, Yale University Medical School,
New Haven, Connecticut 06510

Although properties of the intramembranous charge movement in frog skeletal muscle have now been fairly well characterised[1–5], the function of this process remains uncertain[6–9]. Schneider and Chandler[1] first suggested that the observed charge movement in sartorius fibres held near slack length in a hypertonic paralysing solution took place within the transverse (T) tubular membranes in the region of the triad feet and signalled tubular depolarisation to the sarcoplasmic reticulum (SR), thereby initiating calcium release. More recently it has been reported that much of this charge movement is abolished in semitendinosus fibres stretched well beyond slack length in hypertonic Ringer solution[10]. Other studies using intracellular calcium indicators, however, have shown that substantial amounts of calcium are released by brief depolarisations in muscle fibres stretched to sarcomere lengths of 4.0 μm or more, when bathed in isotonic solutions[11–13]. The question thus arises, is charge movement abolished in muscle fibres stretched in an isotonic Ringer solution? As calcium release persists in these conditions, an inhibition of charge movement would make involvement of this phenomenon with calcium release very unlikely. We report here the influence of stretch on charge movement and on an aspect of mechanical activation indicative of calcium release using standard, microelectrode voltage-clamp experiments on intact frog twitch fibres. We found no dramatic dependence of these properties on fibre length, and the possibility of a functional role for charge movement in the regulation of calcium release remains.

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* Present address: Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907.

† To whom reprint requests should be addressed at: Department of Biology G/5, University of Pennsylvania, Philadelphia, Pennsylvania 19104.

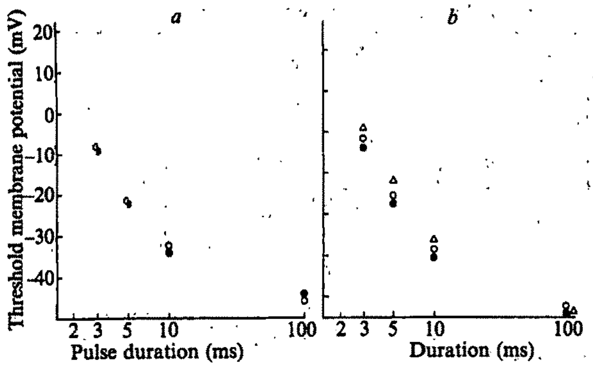

Fig. 1 Strength–duration curves for threshold mechanical responses in voltage-clamped twitch fibres at different sarcomere lengths. Membrane potential at threshold is plotted against duration of the depolarising voltage clamp pulse. Holding potential, −80 mV; temperature, 9–10 °C throughout. *a*, *Rana temporaria* sartorius muscle. Sarcomere lengths: ○, 2.5–2.78 μm; ●, 2.0–2.42 μm, mean values for eight fibres. *b*, *Rana pipiens*. ●, mean values from six sartorius fibres 2.27–2.50 μm long ○, △: Means from six semitendinosus fibres at 2.97–3.07 μm (○) and 3.33–3.48 μm (△) (data obtained by L. L. Costantin on isolated, single fibres).

Threshold mechanical responses to relatively brief (<100 ms) depolarisations in a voltage-clamped muscle fibre are a functional, although indirect, indication of the voltage dependence of calcium release[6,14–16]. The method has been described in detail elsewhere[5,15]. A muscle fibre was visualised under a water immersion objective and impaled with two microelectrodes to measure the intracellular voltage and to pass current through a voltage clamp feedback loop. Sarcomere shortening in response to depolarising voltage clamp pulses of various durations was observed at the surface of the muscle fibre, and the membrane potential at which shortening was just detectable is defined as threshold. Mechanical threshold increases with decreasing pulse duration, so that a brief pulse must be much larger than a long one to bring the myoplasmic calcium level up to threshold.

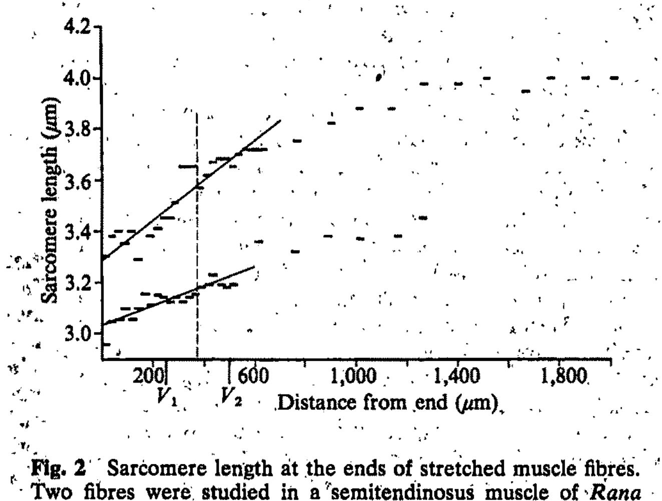

Fig. 2 Sarcomere length at the ends of stretched muscle fibres. Two fibres were studied in a semitendinosus muscle of *Rana pipiens* mounted exactly in the same way as for an electrophysiological experiment. The distance spanned by 10 sarcomeres was measured with a ×10 Filar micrometer (×40 water immersion objective) and divided by 10 to yield sarcomere length. This value is plotted as a bar over the length of measurement. V_1 and V_2 indicate positions of the intracellular voltage sensing micro-electrodes for charge movement experiments; current was sampled over the terminal 375 μm (vertical dotted line). Solid lines drawn by eye.

Figure 1*a* shows strength–duration (*S–D*) curves for threshold contractions in sartorius fibres from *Rana temporaria* at 9 °C. Means from two populations of fibres at different sarcomere lengths (2.0–2.4 μm and 2.5–2.8 μm) are shown. Long pulse thresholds represent a steady state balance between release and re-uptake of calcium by the SR, while short pulse

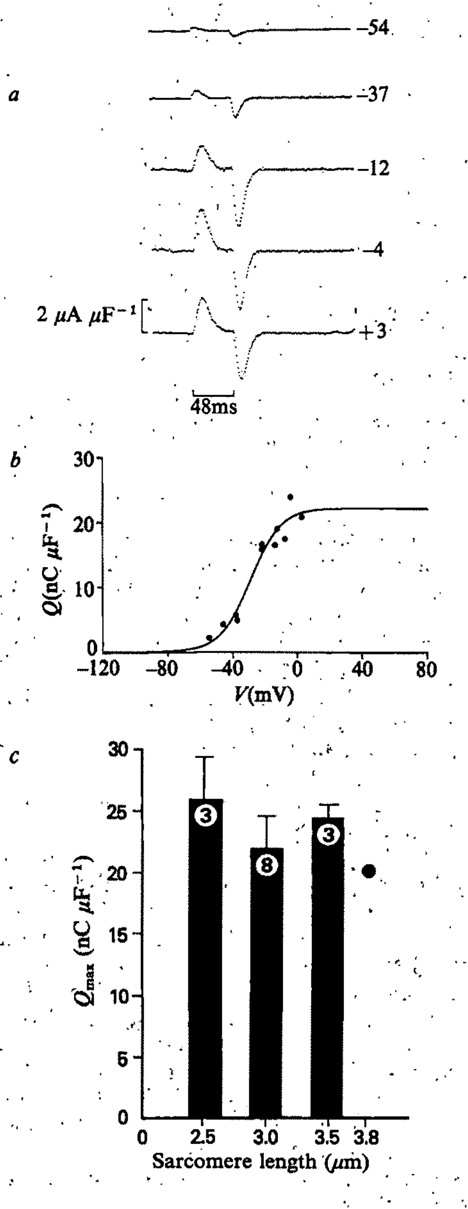

Fig. 3 Charge movement in stretched twitch fibres. *a*, Membrane current ($\Delta V = V_1 - V_2$) for depolarisation to the voltages, in mV, as indicated. Bathing solution contained 115 mM TEA-Cl, 2.5 mM KCl, 11.8 mM $CaCl_2$, 1 mM PIPES, and 2 mM tetracaine. Electrodes spaced 250 μm apart (see Fig. 2); R_i assumed to be 255.2 Ω cm: 1 μA μF⁻¹ corresponds to 1.3 mV of ΔV. Fibre OCT23-78-F4; sarcomere length, 3.0 μm; 7 °C; holding potential −80 mV; feedback control taken from V_2. Uppermost trace is the average of eight sweeps; others are averages of four sweeps. See refs 2, 5 for details of measurement. *b*, Steady state *Q–V* plot for the fibre in (*a*). The points were obtained from the time integrals of current traces, some of which are shown in (*a*). The smooth curve was drawn according to the equation[1]

$$Q(V) = Q_{\max}\left[1+\exp\left(-\frac{V-\bar{V}}{k}\right)\right]^{-1}$$

and was fitted to the points by least squares. The best fit parameters were $\bar{V} = -28.6$ mV, $k = 9.3$ mV, $Q_{\max} = 22.1$ nC μF⁻¹. *c*, Maximum moveable, nonlinear charge, $Q_{\max}$, as a function of sarcomere length at the V_1 electrode. error bars indicate one standard deviation; number of fibres is indicated. Point at 3.8 μm is from one measurement at +5 mV.

thresholds (<10 ms) are probably set by the voltage-dependent kinetics of some regulatory step preceding calcium release. Values at all durations are indistinguishable in these two groups of fibres, suggesting no functional impairment of calcium release by this amount of stretch.

A somewhat greater length range was investigated in *Rana pipiens* sartorius and semitendinosus (single) fibres (Fig. 1*b*). Data from single fibres were obtained by the late Dr L. L. Costantin and have been tabulated previously[15]. Only the means for 2 ms of the 2.27–2.50 and 3.33–3.48 μm populations are significantly different at the 95% level by *t*-test. Again, a major influence of stretch on calcium release is not apparent.

To study membrane charge movements in intact fibres it is necessary to abolish contraction in response to depolarisation. We used an isotonic paralysing solution containing 2 mM tetracaine. Almers and Best[6] have shown that this drug does not interfere with charge movement, and probably directly inhibits the calcium release process in the SR membranes. The solution also contained 115 mM tetraethylammonium (TEA) chloride and 5 mM RbCl to block K currents, tetrodotoxin to block Na currents, 11.8 mM $CaCl_2$ to minimise leakage conductance around the electrodes, and 1 mM PIPES buffer (*p*H 7.0). Temperature was 7 °C.

The three electrode voltage-clamp technique used allows an approximate measurement of membrane current density at the end of a muscle fibre[17]. Feedback control was taken from the electrode more distant (500 μm) from the fibre end (V_2), and the current trace was integrated to determine capacitance as described by Schneider and Chandler[18]. Pulse duration was 48 ms; control pulses were obtained over the range of −80 to −150 mV (refs 1, 2). Membrane current amplitude and the quantity of charge were normalised by the effective membrane capacitance, to be in units of $\mu A\ \mu F^{-1}$ and $nC\ \mu F^{-1}$, respectively. This removes the dependence of charge movement measurements on fibre radius, internal resistivity (R_i), and inter-electrode spacing.

Inhomogeneity of sarcomere spacing at the fibre's end[19] adds a complication to these experiments, however. As current is effectively sampled over the last several hundred micrometres of fibre length, a large number of short sarcomeres at the very end might mask any effect of stretch. To check this, sarcomere spacing was measured at the ends of two semitendinosus fibres in a bundle pinned out in the same manner as for a voltage-clamp experiment. Results are shown in Fig. 2. In both cases a substantial difference in sarcomere length does exist between the end and the middle of the fibre. Over the region in which current was measured in these experiments (the terminal 375 μm), however, there is only a 5–10% decrease in sarcomere length. Charge movement measurements are referred to the sarcomere length measured at the V_1 electrode, 250 μm from the fibre end. It is not likely that an inhomogeneity of sarcomere spacing in this region seriously affects our results.

Figure 3*a* shows examples of charge movement traces at several voltages from a semitendinosus twitch fibre stretched to 3.0 μm sarcomere length. The magnitude of the charge movement increases and the kinetics become faster as the pulses get larger. Figure 3*b* shows the amount of charge moved during each pulse. The solid curve is drawn according to the usual Boltzmann distribution model[1] with a steepness of *e*-fold for 9 mV and a saturating Q_{max} value of 22 $nC\ \mu F^{-1}$. The shape of this Q–V curve is very similar to that obtained from fibres at rather shorter lengths in both isotonic[6] and hypertonic[2,3] solutions, although hypertonicity appears to shift the curve 15–20 mV in the negative direction[6].

Figure 3*c* summarises results from semitendinosus twitch fibres at different degrees of stretch. The total amount of moveable charge, Q_{max}, does not significantly depend on sarcomere length. The point at 3.8 μm is for a single pulse to +5 mV, immediately after which the fibre broke.

While Q_{max} and the steepness of the Q–V distribution do not appear to depend greatly on stretch (see also ref. 20), these are steady state properties, and it remains possible that stretch does slow the kinetics of charge movement. This could be due to a real effect of stretch on the process generating the charge movement or to an indirect effect on some cable properties of the muscle fibre. The records in Fig. 3*a* are not obviously slower than other published records; additional experiments are required to study more carefully the effect of stretch on charge movement kinetics. Any effect of stretch on kinetics might also be augmented in a hypertonic Ringer solution.

Neither the properties of charge movement nor of mechanical activation we measured in fibres in isotonic solutions are significantly affected by stretch to sarcomere lengths at which the contractile filaments no longer overlap. These observations are consistent with the possibility that charge movement in skeletal muscle has a role in regulating calcium release.

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Renal brush border membrane adaptation to phosphorus deprivation in the *Hyp*/Y mouse

H. S. Tenenhouse & C. R. Scriver

Medical Research Council Genetics Group, Human Genetics Center, (McGill University) and De Belle Laboratory for Biochemical Genetics, McGill University-Montreal Children's Hospital, Research Institute, 2300 Tupper Street, Montreal, Quebec, Canada H3H 1P3

Human X-linked hypophosphataemia (XLH)[1] involves a partial loss in net renal reabsorption of phosphate anion (P_i). The mutant male hemizygote has about half the normal renal transport activity[2] and this is quite insensitive to circulating parathyroid hormone (PTH)[2,3]. The renal defect is sufficient to explain persistent hypophosphataemia in XLH. The hypophosphataemic mouse (*Hyp*)[4] also has an X-linked defect in renal reabsorption of P_i (refs 4–7). The mutant male hemizygote (*Hyp*/Y) has about half the normal renal reabsorption activity and this abnormality persists after parathyroidectomy[7]. Conservation of the X chromosome during mammalian evolution and evidence for functional homology between species for specific X-linked genes[8] have encouraged us to use the *Hyp* mouse as a model for the renal defect in XLH. We have studied P_i transport in purified renal brush border membrane vesicles (BBMV) prepared from *Hyp*/Y mice[5,9]. A selective loss involving almost half the Na^+-dependent P_i co-transport activity is

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present in *Hyp*/Y membranes. These findings, genetic studies in man[2,10] and recent physiological evidence[11] suggest that P_i transport in the mammalian nephron occurs by at least two processes: one under the control of an X-linked gene that is more sensitive to PTH inhibition and another process, controlled by an autosomal locus, that is less sensitive to the hormone. We report here on the ability of the *Hyp*/Y mouse to adapt to phosphorus deprivation; the response is observed both *in vivo* in net reabsorption of P_i and *in vitro* in BBMV transport of P_i.

Hyp mice were bred and reared in our laboratory. Adult (>100 d old) *Hyp*/Y and normal +/Y littermates were given either a control diet containing 0.4% (w/w) phosphorus or a low-P diet containing 0.03% phosphorus. Both diets contained the same amount of calcium (0.4% w/w). The animals were not thyroparathyroidectomised. After 2 weeks, mice were placed in Gelman metabolic cages; blood and urine was collected and analysed for calcium, phosphorus and creatinine content measured as before[5].

Table 1 shows that in both *Hyp*/Y and +/Y mice there was *in vivo* renal adaptation to phosphorus deprivation, whereas calcium excretion increased, as expected[12]. Renal adaptation did not fully correct the mutant phenotype: *Hyp*/Y animals, in the adapted state, maintained lower ($P < 0.001$) plasma levels and a higher ($P < 0.001$) fractional excretion of phosphorus than +/Y mice in the adapted state.

Purified BBMV were prepared from *Hyp*/Y and +/Y renal cortex by the method of Booth and Kenny[13] and incubated as before[5,9]. Net uptake of $^{32}P_i$ (100 μM) by BBMV was measured after 15 or 30 s incubation in the presence of NaCl or KCl gradients. The simultaneous uptake of D-glucose (20 μM) was also measured and Na^+/glucose co-transport was used as an internal control. D-glucose and P_i at their respective concentrations did not interfere with the co-transport of each other, and the possibility of significant binding of phosphate to BBMV was excluded by the appropriate osmotic experiments in a sucrose medium[9].

P_i transport activity, both absolute and normalised to simultaneous D-glucose uptake, was significantly greater ($P < 0.001$) in BBMV prepared from *Hyp*/Y and +/Y mice adapted to phosphorus deprivation than in BBMV prepared from animals fed the control diet (Table 2). The characteristic deficiency of Na^+/P_i co-transport activity in *Hyp*/Y membranes was retained in the adapted state. We also examined the kinetics of P_i uptake by adapted BBMV, and found that although there was no change in the apparent K_m value, the V_{max} for uptake was increased twofold in the adapted state (Fig. 1).

These findings are of interest for the interpretation of *in vivo* adaptation to phosphorus deprivation, a phenomenon reported earlier for rat[14] and dog[15]. Others have observed that the adapted kidney becomes insensitive to PTH[16], that adaptation is not dependent on the integrity of parathyroid or thyroid glands[17,18] and that, depending on the species, days or weeks are required for the phenomenon to appear[15,18]. The adaptation is a function of the early proximal renal tubule although more distal parts may also be involved[12,19]. Our findings indicate that renal

Table 1 *In vivo* renal adaptation to chronic phosphorus deprivation in +/Y and *Hyp*/Y littermates (mean ± s.e.)

	+/Y mice		*Hyp*/Y mice	
	Control diet ($n = 4$)	Low-P diet ($n = 4$)	Control diet ($n = 4$)	Low-P diet ($n = 4$)
Plasma P_i (mg dl^{-1})	8.33 ± 0.62	5.09 ± 0.84*	3.97 ± 0.19†	1.01 ± 0.14*†
Phosphorus excretion index	0.280 ± 0.021	0.005 ± 0.001*	0.569 ± 0.024†	0.034 ± 0.004*†
Calcium excretion index	0.015 ± 0.0002	0.175 ± 0.003*	0.016 ± 0.0003	0.192 ± 0.004*

Littermate animals were classified as +/Y (normal male) and *Hyp*/Y (mutant hemizygote) according to quantitative and qualitative phenotypic features[4]. Adult mice were given specified diets (ICN) for 2 weeks. Urine was collected for about 3 h, after which blood was obtained from the tail. Samples were analysed as described elsewhere[5]. Total phosphorus content of diets: control, 0.4% (w/w); Low-P, 0.03% determined by chemical analysis according to Trohler *et al.*[17]. Excretion index (analogous to fractional excretion) is derived from the equation: (urine [P_i] or [Ca] × plasma [creatinine])/(plasma [P_i] or [Ca] × urine [creatinine]).

*Different from control-diet animals: $P < 0.001$, Student *t*-test.

†Different from +/Y animals on corresponding diet: $P < 0.001$, Student *t*-test.

Table 2 Uptake of ^{32}P and ^{14}C-D-glucose by renal brush border membrane vesicles from adult control (+/Y) and hypophosphataemic (*Hyp*/Y) male mice

	+/Y		*Hyp*/Y	
	Control diet (0.4%P)	Low-P diet (0.03%P)	Control diet (0.4%P)	Low-P diet (0.03%P)
P_i uptake (30 s) (pmol per mg protein)				
In KCl	81.4 ± 7.4	67.8 ± 8.9	60.7 ± 8.6	55.7 ± 3.8
In NaCl	807.8 ± 55.8	1,596.2 ± 84.7*	617.4 ± 31.6†	902.5 ± 82.3†*
Normalised P_i uptake (30 s; Na dependent) (pmol P per pmol D-glucose)	9.0 ± 0.2	25.2 ± 1.4*	3.6 ± 0.2†	14.5 ± 0.7†*

Net uptake of ^{32}P-labelled orthophosphate (100 μM) was measured at 30 s by BBMV prepared from renal cortex of normal male (+/Y) and hemizygous (*Hyp*/Y) littermates. The membranes were isolated and purified by the method Booth and Kenny[13], and suspended in 300 mM mannitol and 20 mM Tris-HEPES, *p*H 7.4, at a final protein concentration of 2–3 mg ml^{-1}. Uptake was measured by the Millipore technique used previously[5,9]. The reaction was initiated by adding 90 μl of incubation medium containing ^{32}P and ^{14}C-D-glucose (20 μM) to 10 μl of brush border membrane preparation at 20°C. The reaction was terminated by the addition of 4 ml stop solution (100 mM mannitol, 100 mM NaCl, 20 mM Tris-HEPES and 10 mM arsenate *p*H 7.4, 4°C), and filtered immediately through a 0.45-μm Millipore filter. After two rapid additional washes with stop solution the filters were counted in Aquasol-2 in a liquid scintillation counter. Incubation solutions were: (1) 100 mM KCl, 100 mM mannitol and 20 mM Tris-HEPES, *p*H 7.4; (2) 100 mM NaCl, 100 mM mannitol and 20 mM Tris-HEPES, *p*H 7.4. Background counts were subtracted from experimental counts; the former were always less than 15% of uptake at equilibrium in the presence of membranes. Phosphate is not significantly bound to BBMV and is entirely retained as the inorganic anion after uptake[5,9].

*Difference between low P and control diet is significant: $P < 0.001$, Student *t*-test.

†Difference between +/Y and *Hyp*/Y is significant: $P < 0.001$, Student *t*-test. Data are mean ± s.e.m. of five determinations.

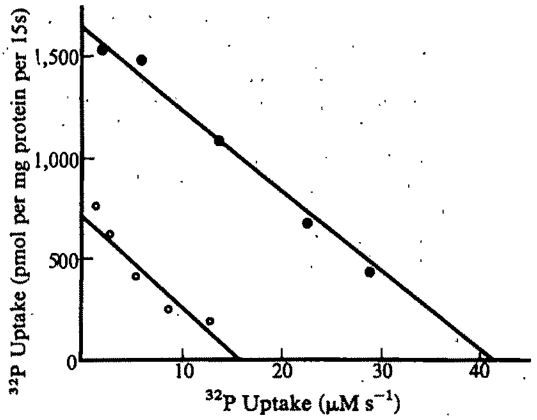

Fig. 1 Eadie–Hofstee plots of the Na$^+$-dependent uptake of ^{32}P at 15 s by BBMV prepared from kidneys of adult normal mice fed control diet (0.4%, w/w) (○) and low-P diet (0.03%) (●), each for 2 weeks. The apparent K_m values are 45 μM in mice fed the control diet and 40 μM in P-deprived mice; V_{max} is increased in the adapted animal (1,659 pmol per mg protein per 15 s compared with 722 pmol per mg protein per 15 s ($P<0.001$)). The correlation coefficients for the regressions were 0.886 and 0.850 for control and P-deprived animals, respectively, over the concentration range 15–800 μM P_i. See Table 2 for details of BBMV incubation and transport assay. Kinetic values refer to Na-dependent uptake exclusively, that is, total uptake in NaCl minus diffusional uptake in KCl.

adaptation to phosphorus deprivation *in vivo* involves a change in P_i transport activity in the brush border membrane. This adaptation occurs in the presence of parathyroid hormone *in vivo* and seems to involve a selective change in the number or activity of carriers specific for phosphate anion. Because adaptation occurs in the *Hyp*/Y animal, we suggest that a brush border membrane mechanism for P_i transport, other than that controlled by the X-linked gene, is responsible for the enhanced BBMV P_i transport activity. The signal for this adaptation is unknown: the hypophosphataemic *Hyp*/Y phenotype itself is insufficient to produce this, and the additional stimulus of environmental phosphorus deprivation is required to evince the membrane responsible in the *Hyp* animal. It is interesting that renal phosphorus content in *Hyp* mice does not differ from normal[5]. We have also observed that phosphorus levels in renal cortex are similar in control and adapted mice[20].

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Is adenosine the mediator of opiate action on neuronal firing rate?

Trevor W. Stone & Martin N. Perkins

Department of Physiology, St. George's Hospital Medical School, University of London, London SW1, UK

Despite recent advances[1,2], relatively little is known of the mechanism of action of opiate analgesics at the cellular level. There is evidence, however, that the inhibition of neurotransmitter release produced by morphine may be mediated by the initial release of adenosine[3–5]; the effect is mimicked by adenosine and the actions of both adenosine and morphine are blocked by theophylline and enhanced by dipyridamole[3–5]. When applied microiontophoretically, adenosine has also been shown to be a potent depressant of the firing rate of single neurones in the mammalian brain, and a similar depressant effect of morphine has been claimed to be stereospecific, antagonised by naloxone[6–9] and greatly reduced in animals made tolerant to, and dependent on, morphine[8,9]. The experiments described here examine the effect of aminophylline, a soluble theophylline derivative, on these depressant effects. We have found that aminophylline will block responses to both morphine and adenosine, suggesting that the depressant responses to morphine may also be mediated by the local release of adenosine.

Male Porton Wistar rats were anaesthetised with urethane, 1.3 g per kg, intraperitoneally. The animals were placed in a stereotaxic frame and the body temperature automatically maintained at 37–38 °C. A burr hole was made so as to allow electrode penetration into the corpus striatum at coordinates according to the atlas of DeGroot[10].

Drugs were applied by microiontophoresis from six- or seven-barrelled micropipettes of tip diameters 4–12 μm. The drugs used were: morphine sulphate, 50 or 100 mM, *p*H 5.5; adenosine hemisulphate, 100 mM, *p*H 4; γ-aminobutyric acid (GABA), 100 mM, *p*H 4; naloxone HCl 50 mM, *p*H 5; aminophylline (theophylline ethylenediamine), 50 mM, *p*H 9 (unadjusted). The latter drug was injected as an anion, and the rest as cations. No retaining current was applied to aminophylline. One barrel of the micropipette contained 165 mM NaCl for current balancing and testing. Action potentials were recorded through another barrel of the multibarrel pipette, filled with 3 M NaCl, or through a separate, single microelectrode glued alongside the multibarrel[11]. The spikes were amplified, gated and displayed on oscilloscopes and the gated pulses were counted and displayed on a polygraph as a record of spikes per s.

The effect of local application of morphine was studied in 127 neurones in the rat striatum using currents of 60–160 nA. Of these, 72 were found to be depressed, 23 excited and 32 did not respond to the alkaloid. The firing of most of the inhibited units was suppressed completely within 1–2 s and recovery from this depression was also rapid, the original firing rate returning within 5–10 s of terminating the ejection current (Fig. 1). On 20 quiescent cells morphine and adenosine also reduced the excitation induced by iontophoretically applied glutamate. The time course of the depression was similar to that of spontaneously firing units.

Adenosine and GABA also depressed neurones responding to morphine (Fig. 1) when applied with currents ranging from 45 to 120 nA. Again, the onset and recovery from these inhibitions were rapid, as has been described previously[12].

The opiate antagonist naloxone was then applied to 29 cells in an attempt to block the responses to morphine. Typically, both inhibitory and excitatory responses were difficult to antagonise, a problem exacerbated by the direct depressant effect of naloxone itself. However, 14 cells did show a reduction of morphine responses whereas GABA responses were unaffected, although recovery from naloxone blockade was slow.

The effects of aminophylline on morphine responses has been tested on 15 neurones. Nine of these exhibited some increase of

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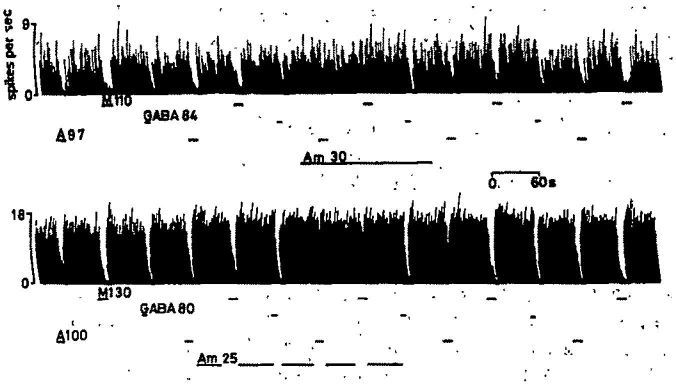

Fig. 1 Ratemeter records of the firing rate of two spontaneously active neurones in the rat striatum. The firing of both cells was depressed by the application by microiontophoresis of adenosine (A), morphine (M) and GABA. The application of aminophylline (Am) as an anion rapidly blocked the responses to adenosine and morphine, whereas GABA responses were unaffected. The times of drug application are shown by bars below the records, and the figures are the iontophoretic currents used, in nA. Time bar, 60 s.

baseline firing rate during the ejection of aminophylline. In all 15 cells the depressant responses to morphine were rapidly and reversibly antagonised by doses of aminophylline ranging from 14 to 90 nA, and an example of this is shown in Fig. 1. Depressant responses to adenosine were always greatly reduced or abolished in parallel with the loss of morphine responses, whereas the effects of GABA remained unaffected (Fig. 1). Ethylenediamine had no effect on the agonist responses.

These experiments demonstrate a powerful depression by morphine of the firing rate of neurones in the rat corpus striatum, an area which was chosen for this study because it contains a high density of opiate receptors[13,14]. The ability of naloxone to antagonise the morphine depression of firing supports the view expressed by others that such depressant responses result from an action on the specific opiate receptor[6–9], although the difficulty experienced with naloxone may result from an action of morphine on several classes of receptor, as discussed by Lord *et al.*[15].

The inhibitory effect of iontophoretically applied adenosine has been previously described as a widespread phenomenon in the central nervous system[12,16–19]. Furthermore, the effects of this purine in many tissues and preparations can be blocked by methylxanthine derivatives such as theophylline[3–6,12,16–22]. Our demonstration that aminophylline can antagonise responses to both adenosine and morphine but not GABA, while the effects of morphine, but not adenosine, can be blocked by naloxone leads us to propose that the first result of the interaction of morphine with its receptor is a release of adenosine from the tissue. Such a sequence presumably also explained the inhibition of transmitter release by morphine and its modulation by dipyridamole and theophylline reported by other workers using peripheral[3,4] or central[5] models of transmitter release. Indeed, Fredholm and Vernet[23] have now shown directly that morphine will enhance the depolarisation-induced release of adenosine from neurones[23].

It could be argued that, as adenosine and morphine are potent inhibitors of calcium-dependent neurotransmitter release, such an action could contribute to their depressant effects on neuronal firing. Aminophylline might then reduce that action by increasing calcium availability in the presynaptic terminals. However, the only relevant work seems to be that of Edstrom and Phillis[24], who reported that adenosine hyperpolarised cortical neurones without affecting membrane conductance, although this finding, as the authors noted, does not prove a presynaptic site of action. On the other hand, we have demonstrated that the depressant effects of morphine and adenosine occur against excitatory responses to glutamate, an amino acid thought to act postsynaptically by increasing sodium conductance[25].

We also suggest that the release of adenosine may be highly relevant to the analgesic effects of morphine in the conscious animal, as morphine analgesia has been shown to be reduced by the systemic administration of theophylline[26]. Furthermore, Horrobin has claimed that adenosine is a potent antagonist of many of the effects of prostaglandins[27,28]. A release of adenosine by opiates as suggested here may therefore explain the antagonism by morphine of prostaglandin-induced increases in cyclic AMP[29,30], an action which Collier has proposed to be central to an understanding of opiate tolerance and dependence[31].

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Vanadate inhibits uncoupled Ca efflux but not Na–Ca exchange in squid axons

R. DiPolo*, H. R. Rojas* & L. Beaugé†

* Laboratorio de Permeabilidad Iónica, Instituto Venezolano de Investigaciones, Científicas, Caracas, Venezuela
† Physiological Laboratories, Cambridge, UK

Nerve cells can maintain a very low intracellular calcium concentration ($[Ca^{2+}]_i$) against large Ca^{2+} electrochemical gradients (see ref. 1 for review). The properties of the calcium efflux from these cells depend on $[Ca^{2+}]_i$ (ref. 2), and within the physiological range, most Ca efflux depends on ATP (which stimulates with high affinity) and is insensitive to Na_i, Na_o and Ca_o (uncoupled Ca efflux). When the $[Ca^{2+}]_i$ is well above the physiological range, Ca efflux becomes only partially dependent on ATP (acting now with low affinity), is inhibited by Na_i and is stimulated by Na_o and Ca_o (Na–Ca exchange). Orthovanadate, a powerful inhibitor of the $(Na^+ + K^+)$ATPase and the Na pump[3,4], also inhibits the Ca-stimulated ATPase activity, which is the enzymatic basis for the uncoupled Ca pump, in human red cells[5]. The experiments reported here show that in squid axons the ATP-dependent uncoupled Ca efflux can be fully and reversibly inhibited by vanadate, whereas concentrations of vanadate 10 times higher have no effect on the Na–Ca exchange. This is another indication that the uncoupled Ca efflux represents an ATP-driven Ca pump, and supports the suggestion that the uncoupled Ca efflux and Na–Ca exchange are mediated by different mechanisms[2].

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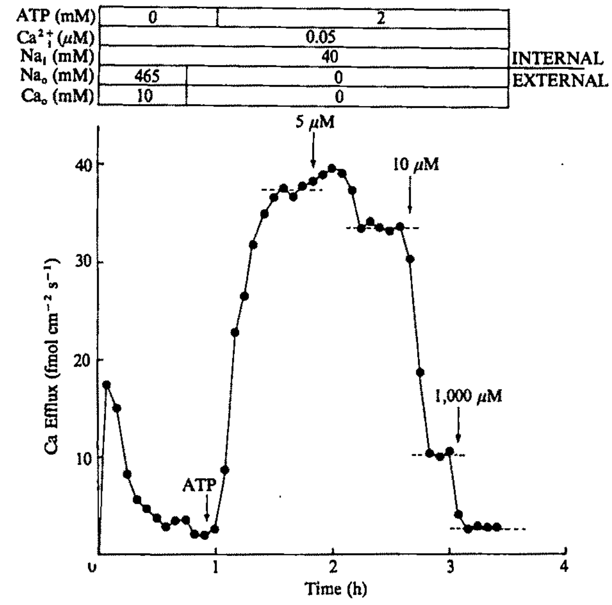

Fig. 1 Effect of different internal vanadate concentrations on Ca efflux in an axon dialysed with 0.05 μM $[Ca^{2+}]_i$ (axon 150379, 400 μM). The composition of the dialysis solution was (mM): K^+, 310; Na^+, 35; Mg^{2+}, 4 in excess to the ATP concentration; $Tris^+$, 5; Cl^-, 79; aspartate, 310; EGTA, 1; glycine, 330; and oligomycin, 10 μg ml^{-1}; at 980 mosmol, *p*H 7.1 (17 °C). ATP (Boehringer) and phosphoarginine (PA) (Calbiochem) were used as sodium salts and were stored at −90 °C as stock 250 mM solutions at *p*H 7.1 adjusted with Tris-OH (ATP) and HCl (PA). The ATP solution also contained 250 mM $MgCl_2$. The composition of the standard artificial seawater was (mM): Na^+, 465; K^+, 10; Mg^{2+}, 50; Ca^{2+}, 10; $Tris^+$, 10; Cl^-, 580; EDTA, 0.1; 1,000 mosmol, *p*H 7.6 (17 °C). Tris was used as a substitute for Na. Removal of Na_0 and Ca_0 was carried out without changing the other constituents. The nominally Ca-free seawater also contained 0.5 mM EGTA and no added Ca. The axon was incubated for 1 h before dialysis in artificial seawater containing 1 mM KCN. The arrows refer to addition of vanadate and the concentration is indicated. The flow of the dialysis solution was 1 μl min^{-1}. The flow of external solutions was about 1 ml min^{-1} and was collected at 5-min intervals, added to 5 ml of scintillator and counted. For more details of the dialysis technique see ref. 6.

The experiments were carried out on giant axons taken from live specimens of the tropical squid *Dorytheutis plei.* The intracellular concentration of low molecular weight solutes was controlled using the internal dialysis technique[6]. To inhibit endogenous ATP production, the axons were soaked in seawater containing 1 mM KCN for 1 h before the dialysis began. The production of ADP from the hydrolysis of exogenous ATP was minimised by adding 5 mM phosphoarginine to the dialysis solutions.

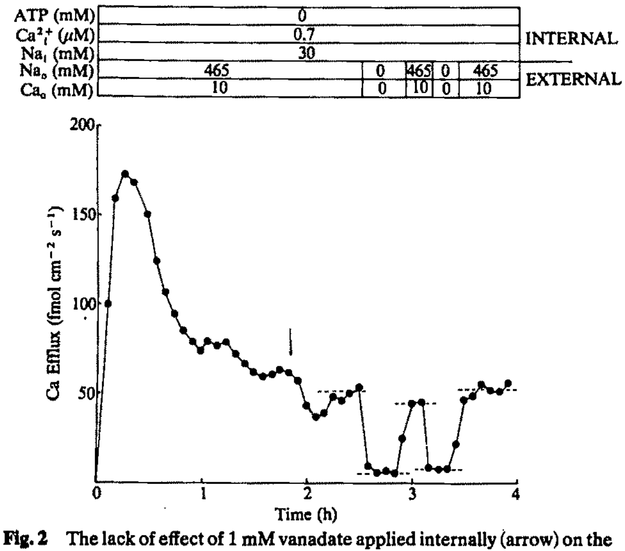

Fig. 2 The lack of effect of 1 mM vanadate applied internally (arrow) on the Na–Ca exchange in squid giant axon (axon 190179 B, 450 μm). In this experiment the axon was dialysed with a solution lacking ATP (to inhibit the uncoupled Ca efflux) and with 0.7 μM $[Ca^{2+}]_i$ to maximise the Na–Ca exchange component. The general technique was the same as that described in Fig. 1 legend.

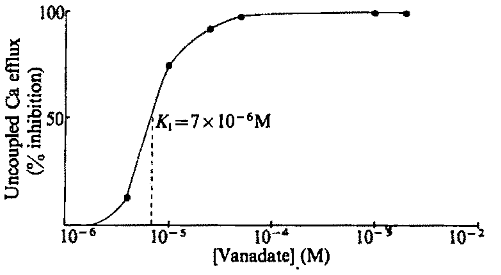

Fig. 3 Dose-response curve for the vanadate inhibition of the ATP-dependent 'uncoupled' Ca efflux in squid giant axons. The general technique was similar to that in Fig. 1, where after a steady Ca efflux was obtained in the presence of ATP, different concentrations of vanadate were tested. In all cases the external solutions lacked both Na and Ca. Each point is the mean of three axons. The inhibitory effect of vanadate was always reversible. ATP, 2 mM; Na_i, 40 mM; K_0, 10 mM; 17 °C.

Figure 1 shows that in an axon dialysed with 0.05 μM Ca^{2+} the ATP-dependent 'uncoupled' Ca efflux is progressively inhibited by increasing amounts of vanadate applied internally. The lack of effect of vanadate on the Na–Ca exchange is readily demonstrated in the experiment shown in Fig. 2. In this case the axon was dialysed with a solution free of ATP and containing 0.7 μM Ca^{2+} to maximise the Na–Ca exchange[2]. The addition of 1 mM vanadate did not significantly change the levels of Ca efflux. (The small reduction seen could be due to inhibition of an uncoupled Ca efflux caused by ATP remaining inside the axon.) In addition, the removal of Ca_0 and Na_0 reversibly inhibited Ca efflux, bringing it to leakage levels. Both the absolute magnitude of the Na_0–Ca_0-activated Ca efflux and the leakage levels are similar to those found in axons not poisoned with vanadate, dialysed with the same $[Ca^{2+}]_i$. Results similar to those shown in Figs 1 and 2 were seen in three other experiments. Figure 3 is a dose-response curve for the vanadate inhibition of the ATP-dependent uncoupled Ca efflux in axons dialysed with 0.05 μM Ca^{2+}. From the curve, the K_i for vanadate is about 7×10^{-6} M. The inhibition by vanadate was always reversible.

If one accepts the possibility that the Ca pump is widespread in tissues and species[7], the vanadate inhibition of the Ca-ATPase activity in red cells and the ATP-dependent Ca efflux in squid axons could be taken as a strong suggestion that they are due to an identical process.

It is intriguing that whereas ouabain selectively inhibits the Na–K pump, vanadate inhibits both the Na–K and the Ca pumps. Vanadate almost certainly inhibits the Na–K pump by slowing the conformational change that precedes the release of K from the enzyme–potassium complex, $E_2(K)$, formed after dephosphorylation (refs 8, 9 and L. B. Karlish and I. M. Glynn, unpublished); in addition, external K is required for inhibition[8–10]. There is no indication as to the mechanism for vanadate inhibition of the Ca–ATPase, although Na^+ and Mg^{2+} are known to facilitate this inhibition[5]. The present experiments show that, similar to its effect on $(Na^+ + K^+)$ATPase, vanadate inhibits the Ca pump by acting at the intracellular surface. On the other hand, this inhibition was not dependent on external K (data not shown). Experiments are in progress to determine the sidedness of vanadate and cation interactions in the inhibition of the Ca pump in squid axons.

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A selectively driven molecular clock

Daniel Hartl & Daniel Dykhuizen
Purdue University, Department of Biological Sciences,
West Lafayette, Indiana 47907

The problem of whether mutations that increase a species' fitness are incorporated into the genome as a function of generation interval or as a function of absolute time is of some interest because of the clock-like nature of amino acid substitutions known to have occurred in the evolution of certain well studied proteins. The molecular clock features a rate of amino acid substitution that is relatively constant when averaged over a sufficiently long time, and the constant rate of substitution is independent of the generation interval of the species involved[1,2]. Furthermore, the variance in the rate of substitution is roughly twice the mean[2]. Some authors have argued that the very existence of a molecular clock implies that most amino acid substitutions must be selectively neutral or nearly neutral[3]. On the other hand, there is no compelling reason to assume that selectively driven gene substitutions would not also exhibit clock-like behaviour, in which case the rate of increase in fitness of a population should be a function of absolute time rather than generation interval. In this report, we have investigated the issue of rate of increase in fitness using initially isogeneic strains of *Escherichia coli* that had been allowed to evolve independently in glucose-limited chemostats[4] for 500 h. The nutrient supply was adjusted in such a manner that some of the strains maintained a doubling time of 2.5 h and others a doubling time of 5.0 h, and the relative fitnesses of the evolving strains were measured periodically. Our main conclusions are that (1) selectively driven gene substitutions detectable as increases in fitness do exhibit clocklike behaviour, (2) when averaged over a sufficiently long time, the rate of increase in fitness is a function of absolute time and independent of generation interval, and (3) except for an initial period related to adjustment to chemostat conditions, the variance in the rate of increase in fitness is very nearly twice the mean.

At each generation interval, two isogeneic strains were maintained in separate chemostats. One strain was DD616, which is sensitive to bacteriophage T5 (T5^S) and which carries *rpsL*, a non-reverting streptomycin-resistance mutation used to verify that the chemostats remained free of contamination; the other strain was selected as a spontaneous mutation in DD616 that confers resistance to T5 (T5^R), a phenotype associated with the *tonA* locus. Each chemostat contained 30 ml of medium and quickly reached an equilibrium density of about 5×10^8 cells per ml. Samples were withdrawn from the chemostats at 100-h intervals and aliquots containing approximately 10^{10} cells were frozen in 20% glycerol at −20 °C for subsequent study.

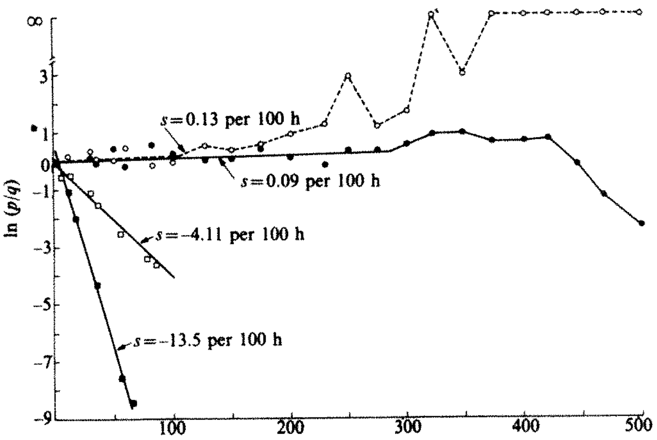

Fig. 1 Changes in the proportion of T5^R cells (p) relative to the proportion of T5^S cells (q) in chemostats. ●, Isogeneic T5^R and T5^S cells maintained at a 2.5-h doubling time. ○, Isogeneic T5^R and T5^S strains maintained at a 5.0-h doubling time. ■, Chemostat with 2.5-h doubling time containing unevolved T5^R strain against T5^S strain that had evolved for 200 h at a 5.0-h doubling time. □, Chemostat with 5.0-h doubling time showing genetic divergence between a T5^R strain grown for 300 h at a 2.5-h doubling time and a T5^S strain grown for 300 h at a 5.0-h doubling time.

The selective difference between any two strains was estimated by inoculating a glucose-limited chemostat with approximately 10^9 growing cells from each strain. The chemostat was maintained at either a 2.5-h or a 5.0-h generation time for 80–100 h, and at intervals the chemostat was sampled and the relative proportions of the two strains ascertained. The genetic marker used to distinguish the strains was resistance or sensitivity to T5, and chemostat samples were either plated on nonselective medium to estimate the total number of cells or were plated in the presence of an excess of T5 to estimate the number of T5^R cells; the ratio of T5^R cells to total cells estimates the proportion, p, of T5^R cells in the chemostat.

Selection in the chemostat is indicated by a systematic increase or decrease in the frequency of T5^R. Indeed, for a haploid population undergoing continuous growth[5], $dp/dt = pqs$, where $q = 1 - p$ represents the proportion of T5^S cells at

Table 1 Fitness differences observed per 100 h for strains that had evolved for various lengths of time in either 2.5-h or 5.0-h chemostats

	2.5-h chemostats	T5^R					
	Time (h)	0	100	200	300	400	500
T5^S	0	0.09±0.06					
	100	−12.82±1.14*	−0.25±0.16				
	200	−15.66±2.83*	−0.44±0.15†	0.34±0.22	1.02±0.28†		
	300		−1.16±0.20*	−0.28±0.21	0.22±0.21	3.48±0.37*	
	400		−5.48±0.24*		−3.89±0.18*	−1.55±0.28*	−0.01±0.20
	500		−5.98±0.20*			−1.90±0.30*	−0.71±0.31
	5.0-h chemostats	T5^R					
		0	100	200	300	400	500
T5^S	0	0.13±0.23					
	100	−9.63±0.65*	−2.11±0.27*				
	2Q0	−12.90±0.33*	−9.81±0.52*	−0.07±0.24	1.47±0.70		
	300		−11.91±0.21*	−3.60±0.26*	−1.70±0.13*	0.27±0.11	
	400		−10.10±0.40*		−2.31±0.31*	−1.10±0.17*	−0.23±0.30
	500		−10.94±0.29*			−0.88±0.15*	−1.42±0.17*

In each case the selection coefficient is that of T5^R relative to T5^S. Underlined values are means of two independent estimates that were not significantly different. All selection coefficients are estimated by the slope of a regression line, as shown in Fig. 1. * and † indicate values significantly different from 0 at 1% and 5% levels, respectively.

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time t, and s is the selection coefficient denoting that e^s is the rate of increase in the number of $T5^R$ cells relative to a value of 1 for the rate of increase of $T5^S$. Thus, $\ln(p_t/q_t) = \ln(p_0/q_0) + st$, so a plot of $\ln(p/q)$ against t is linear with slope s.

Figure 1 shows the selective equivalence of the original, unevolved $T5^R$ and $T5^S$ strains when grown in chemostats at 2.5 h (filled circles) or 5.0 h (open circles). These control chemostats were established at the same time as the experimental ones and were identical to them, except that the controls were inoculated with equal numbers of $T5^R$ and $T5^S$, and the frequency of $T5^R$ was periodically estimated. In the 2.5-h chemostat the frequency of $T5^R$ remained at very nearly 50% for 275 h; the slope of the linear regression from 0 to 275 h is 0.09/100 h and is not significantly different from 0. After 275 h the frequency of $T5^R$ begins to change somewhat erratically, a phenomenon caused by periodic selection[6]—the chance occurrence of rare, favourable mutations that carry the associated T5 marker with them as they change in frequency. The onset of periodic selection in the 5.0-h chemostat is earlier (125 h) and more pronounced, but the initial slope of the regression (0.13/100 h for 0–100 h) is again not significantly different from 0.

Results of the experiments are summarised in Table 1, which presents the selection coefficient in favour of $T5^R$ in competition experiments involving $T5^R$ and $T5^S$ strains that had evolved independently for various lengths of time. The upper and lower matrices refer, respectively, to strains evolved and tested at a 2.5-h and a 5.0-h generation interval. In these matrices the difference between any number and the number immediately below it represents the increment in fitness achieved in the corresponding 100 h of evolution; 100-h increments in fitness are also given by the difference between any number and the number immediately to its left.

Fitness increments calculated from Table 1 are presented in Table 2. Those increments which did not differ significantly from 0 were entered as 0 in Table 2. (The two negative entries in Table 2 are probably spurious, as the probability of obtaining one or more spurious significances at the 1% or 5% level with 44 tests is 36% and 90%, respectively.) As indicated in Table 2, considerable variation in the fitness increments occurs. The large values obtained for 0–200 h undoubtedly arise from the selection of favourable mutations present in the initial inoculum of 2×10^9 cells. The somewhat more regular behaviour of the fitness increments in the period 200–500 h represents long-term adjustment to the chemostat based on incorporation of newly arising mutations as evidenced by periodic selection in the controls. The overall average fitness increment per 100 h is 2.99 in the 2.5-h chemostat and 3.00 in the 5.0-h chemostat. In the 200–500-h period, the average fitness increments per 100 h are 1.55 and 1.10 in the 2.5-h and 5.0-h chemostats, respectively; these values are not significantly different.

Table 2 Fitness increments during various intervals of evolution in chemostats having either a 2.5-h(*a*) or a 5.0-h(*b*) doubling time

	0–100	100–200	200–300	300–400	400–500
a					
	12.91	0	0.72	4.32	0
	12.57	0	0	4.11	0
	15.22	0.78	0.80	5.03	0
		0.88	0	3.26	1.54
			0	2.34	1.19
	13.57	0.42	0.30	3.81	0.55
b					
	9.76	3.27	2.10	−1.81	0
	7.52	7.70	3.53	0	0
	3.09	9.74	3.17	1.37	1.19
		8.31	1.54	1.97	0.87
			1.90	1.21	−0.54
	6.79	7.26	2.45	0.55	0.30

Values were obtained from Table 1 by subtracting an entry from either the one immediately above it or the one immediately to its right. Differences not significantly different from 0 are entered as 0. Values beneath each column are unweighted means.

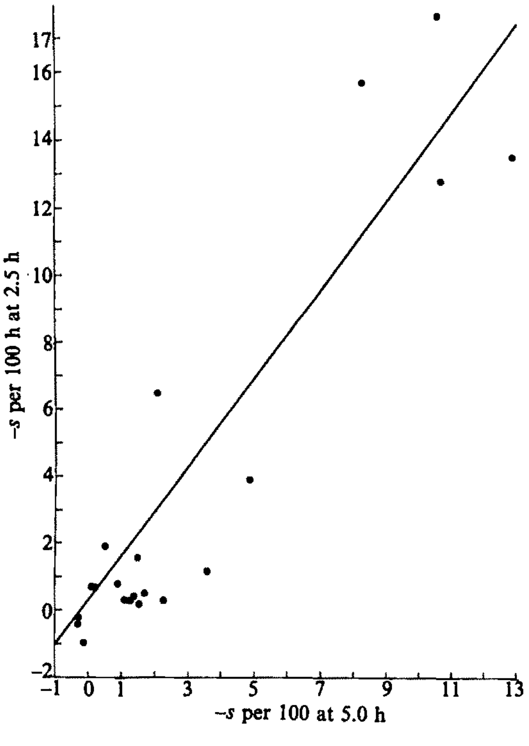

Fig. 2 Relationship between the observed fitness difference of two strains at a 2.5-h doubling time and the observed fitness difference of the identical strains at a 5.0-h doubling time. The regression is $y = 0.29 + 1.35x$.

Thus, when averaged over a sufficiently long time, the rate of increase in fitness under selection is constant and independent of generation time. However, the comparisons in Table 2 would be invalid if a given fitness increment at 2.5 h were magnified to twice its value at 5.0 h. In the worst case the regression of fitness increment at 2.5 h on fitness increment at 5.0 h would have a slope of 0.5, for then the values in Table 2*a* would have to be corrected by multiplying by a factor of 2, and the average fitness increments would then be a function of generation interval.

Figure 2 shows 21 cases where two strains were competed at both 2.5 and 5.0 h. The regression is $y = 0.29 + 1.35x$, where y and x represent the selection coefficients at 2.5 and 5.0 h, respectively; the linear regression on x accounts for 85% of the variation in y. The intercept, 0.29, has a standard error of 0.67 and does not differ significantly from 0, as expected. The slope, 1.35 (with s.e.m. 0.14), differs significantly from 0.5 but its 98% confidence interval includes 1. The comparisons in Table 2 that imply a selectively driven molecular clock are therefore justified.

As for the variance in fitness increments, attention should be restricted to the 200–500 h period to exclude the large effects in the first 200 h due to pre-existing mutations in the initial inoculum. For this period, the overall mean of the 30 increments in Table 2 is 1.33 per 100 h and the variance is 2.58. As the variance is very nearly twice the mean, the selectively driven fitness clock is remarkably similar to the molecular clock based on comparisons of amino acid sequences.

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Macromolecular structural transitions in Pf1 filamentous bacterial virus

C. Nave, A. G. Fowler, S. Malsey, D. A. Marvin & H. Siegrist

European Molecular Biology Laboratory, Heidelberg, FRG

E. J. Wachtel

Department of Chemical Physics, Weizmann Institute of Science, Rehovot, Israel

The filamentous bacterial virus Pf1 is a simple model for biological filaments. We have studied the structure of the virion and report here that the helix parameters of Pf1 change sharply with temperature at about 8 °C. Local interactions between protein subunits change by only a few tenths of an ångstrom, but the changes are amplified between one end and the other of the virion to a rotation of 15 turns and a translation of 1,000 Å. The limited nature of the phase transition is probably due to the constraints of 'knobs-into-holes' interaction between side chains of adjacent α-helical protein subunits. Treatment of the virion with ether causes a rearrangement of protein subunits into sheets, with the α-helices normal to the plane of the sheet. This phase transition suggests a model for virion assembly in the bacterial membrane.

The Pf1 virion is a helical array of 46-residue α-helical protein subunits. It is 20,000 Å long by 60 Å in diameter, encapsulating a single-stranded circular DNA molecule at its core. Two types of X-ray fibre diffraction patterns from Pf1 have been obtained[1], both showing the same general distribution of intensity but each showing slightly different layer line positions (Fig. 1). Fibres prepared at room temperature show a pattern of layer lines indicating 4.40 units in one turn of the basic helix pitch, whereas fibres prepared at 4 °C show a pattern of layer lines indicating 4.46 units per turn (*u*/*t*). Although the local difference between the surface lattices of the two structures is slight, the effect is cumulative (Fig. 2), so that over the 20,000 Å length of the virion, the transition causes one end of the virion to rotate with respect to the other by about 15 turns, at the same time causing the length of the virion to change by about 1,000 Å. The same sort of change can be induced in a wet gel. When the gel is prepared at room temperature, it shows features typical of the 4.40 *u*/*t* diffraction pattern; when the temperature is reduced, features typical of the 4.46 *u*/*t* pattern appear within the minimum time necessary to obtain a diffraction pattern. The transition in the gel is reversible. We have varied many parameters other than temperature, including hydration of the fibre, *p*H, ionic strength, nature of monovalent or divalent cations present, and organic solvents, but so far we have found no other treatment that causes this transition at room temperature.

It is convenient to discuss this transition with regard to a specific molecular model. The simplest model of the protein subunit that explains the diffraction data is a gently curved α-helix rod about 70 Å long by 10 Å in diameter with its long

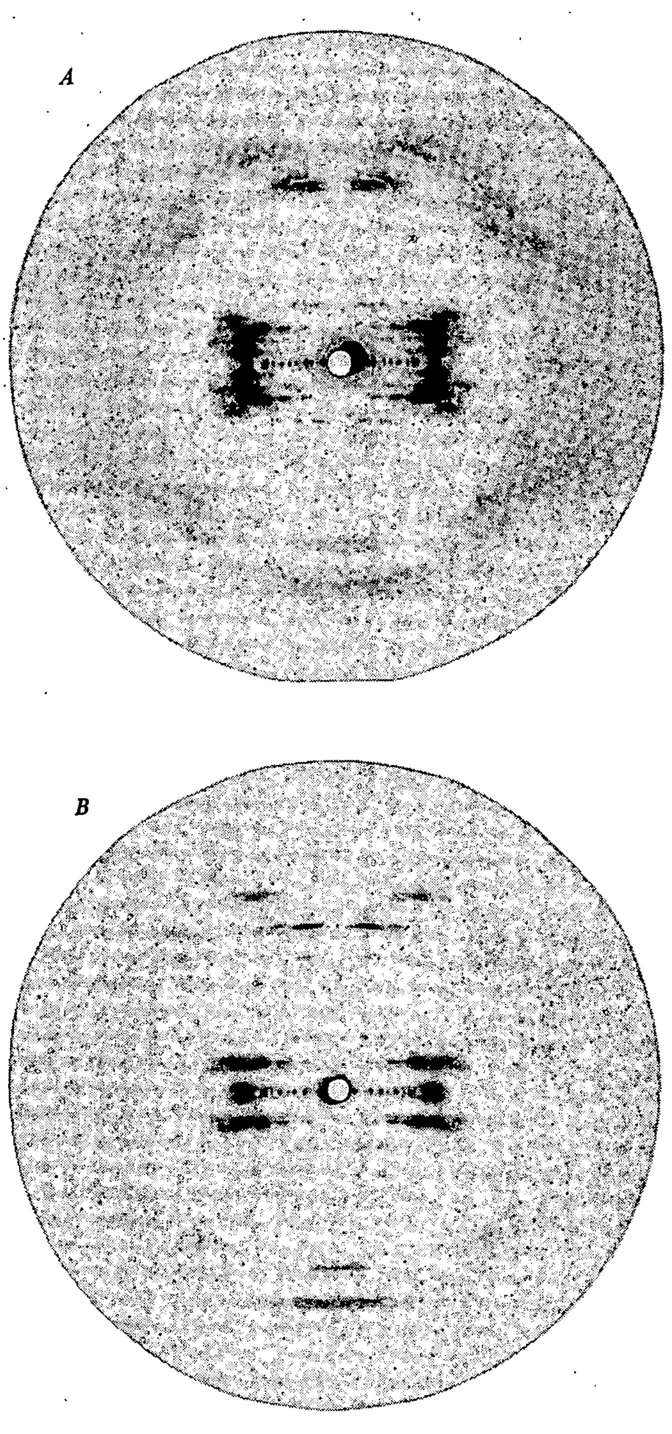

Fig. 1 X-ray diffraction patterns of Pf1 bacterial virus. X-ray fibre diffraction techniques have been described[20]. Fibres were prepared at the temperatures indicated, but X-ray patterns were taken at room temperature after the conformation had been fixed in the dried fibre. A strong magnetic field (6–7 T) was used to improve orientation of the α-helical virions in the fibres, following X-ray diffraction experiments by Samulski[21] and by J. Torbet in collaboration with us (report by Maret *et al.*[22]) showing that α-helices and Pf1 orient parallel to a magnetic field. The fibre axis (and the virion axis) is vertical. The broad distribution of intensity on the pattern gives information about the subunit shape. Strong regions of intensity in the equatorial (horizontal) direction at 10 Å and in the meridional (vertical) direction at 5 Å indicate that most of the protein in the virion is in the form of α-helix with the α-helix roughly parallel to the virion axis. The horizontal lines of intensity (layer lines) give information about the virion symmetry. Near-meridional spacings on layer lines at orders of 0.06385 Å^{-1} in the 4.40 *u*/*t* form and 0.06020 Å^{-1} in the 4.46 *u*/*t* form give the pitch of the basic helix as 15.66 and 16.61 Å, respectively. The positions of the other layer lines give information about the number of equivalent protein subunits in one turn of the basic helix. These other layer lines change position relative to the basic set as a result of the temperature-induced transition. Because of an inherent ambiguity in fibre diffraction patterns, it is not clear[3,4] whether the number of units per turn at room temperature is 4.40 or 5.40, but the detailed interaction between neighbouring subunits is virtually the same for the two alternatives, so this ambiguity does not affect the considerations discussed here. We choose 4.40 *u*/*t* for the purpose of discussion. *A*, 4.40 *u*/*t*, unit rise 3.56 Å, $a = 62.5$ Å, 92% relative humidity, specimen–film distance 7.50 cm, tilt 8°, disorientation half-width 2.3°. Fibre AF 12/2, prepared at room temperature, film 4055, ×0.98. The layer line splitting in the 5 Å meridional region can be approximately explained by a left-hand coiled-coil[5] of radius about 1 Å and pitch 150 Å. *B*, 4.46 *u*/*t*, unit rise 3.73 Å, $a = 61.9$ Å, 98% relative humidity, specimen-film distance 8.28 cm, tilt 12°, disorientation half-width 1.5°. Fibre AF 35/1, prepared at 4 °C, film 4158, ×0.9.

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axis oriented roughly parallel to the virion axis and also sloping radially[2]. Variants of this model have also been discussed[3]. Each subunit is in contact with neighbours 5 units and 9 units along the basic virus helix (Fig. 2). These contacts can be considered by imagining the axes of the α-helices to move smoothly along the 5-start or 9-start directions, so as to sweep out sets of twisted ribbons, or helicoids[2,4].

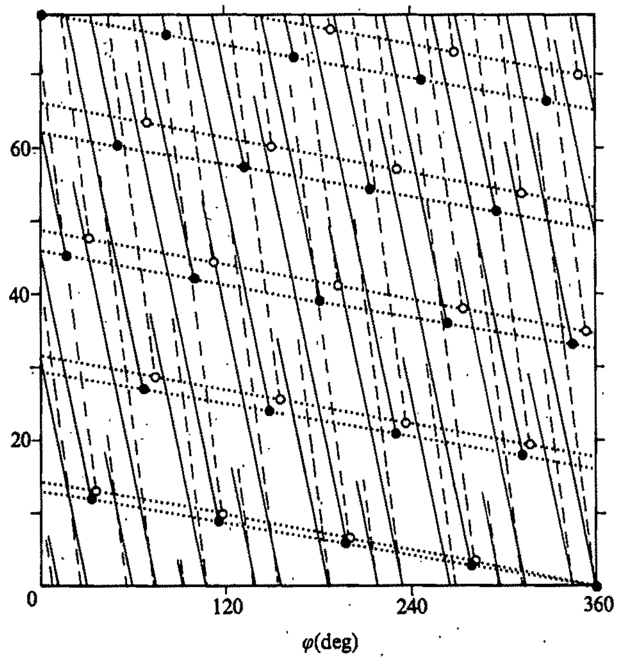

Fig. 2 Surface lattices of Pf1 helices with 4.40 u/t (filled circles and solid lines) and 4.46 u/t (open circles and dashed lines). The dotted lines indicate the basic helices. The surface lattice is generated by projecting equivalent points along radii onto a cylinder of 11.4 Å radius, and then cutting the cylinder vertically to give a two-dimensional representation. The circles represent the projections of equivalent points and the lines represent the projection of the α-helix axes.

Details of the model become less important if we discuss the changes in the structure during the transition rather than the absolute structures. The axis of the subunit in the 4.46 u/t model is more nearly parallel to the axis of the virion than in the 4.40 u/t model (Fig. 2), but the shape of the protein subunits and the interactions between them remain virtually unchanged during the transition. The close relationship between the two structures can be seen by comparing the diffraction data from the strong J_5 and J_9 layer lines of the two structures (Fig. 3*A* and *B*). Although the ζ (vertical) positions of these layer lines change as a result of the transition, because of the change in helix symmetry, the intensity distributions along these layer lines (which reflect the molecular structure of the subunit) show virtually no change. The similarity between amplitudes predicted for the two models (Fig. 3*C* and *D*) is comparable to the similarity between amplitudes observed in the diffraction experiment. Large changes within the subunit, such as bending at a hinge, are not supported by the data. Very small and subtle changes in neighbour–neighbour interactions are sufficient to explain the observations. In particular, the fit of side chains of one subunit into the space between side chains of its neighbours ('knobs-into-holes')[5] is unchanged. This similarity was defined more precisely by calculating distances between pairs of β-carbons on neighbouring subunits where the β-carbons approach one another to within 5 Å. The specific pairs of atoms involved in such contacts do not alter between the 4.40 u/t and the 4.46 u/t models, and even the absolute distances between atoms in these pairs change by only a few tenths of an ångstrom.

The transition appears to be discontinuous and limited. That is, no intermediate forms between 4.40 u/t and 4.46 u/t have been observed, and no other forms have been observed outside these two extremes. The limitation follows directly from consideration of the knobs-into-holes packing in the model. Knobs-into-holes interactions constrain the crossing angle between the axes of neighbouring α-helices[5]. Ideal α-helices with an internal rotation per residue of 100° will cross at an angle of 20°. If the crossing angle changes from 20°, the internal rotation per residue must change correspondingly. However, stereochemical constraints limit the rotation per residue to within a degree or so of 100° (ref. 6), so crossing angles of roughly parallel helices are near 20° (ref. 7). Packing between neighbouring α-helical subunits in the Pf1 model is also constrained by knobs-into-holes considerations[2]. In order to retain unchanged knobs-into-holes packing during the transition from a 4.40 u/t to a 4.46 u/t model, the rotation per residue within the α-helix must be changed by about 0.5°. The absence of virions with less than 4.40 u/t or greater than 4.46 u/t can then be explained as a result of a limitation on internal twist within the α-helix that is coupled by knobs-into-holes packing to the number of units per turn in the virion. This same constraint was shown in a different

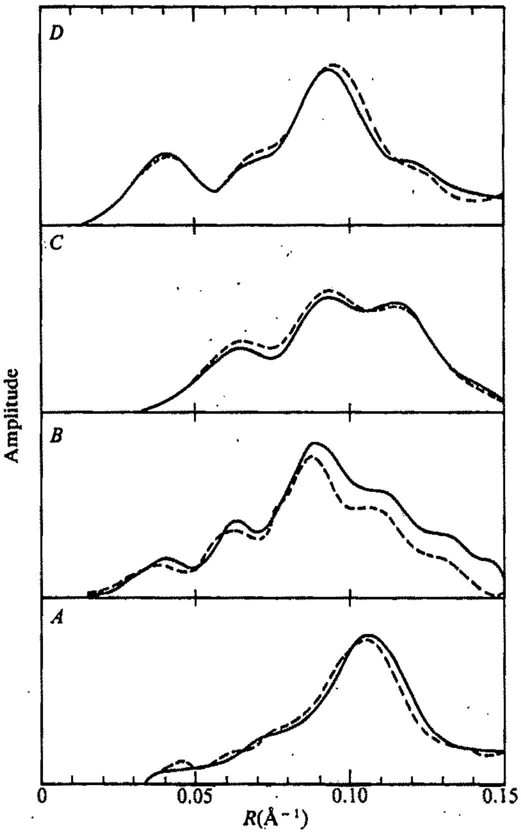

Fig. 3 Distribution of diffraction amplitude along layer lines in the strong 10 Å near-equatorial region. Solid curves represent 4.40 u/t amplitudes and dashed curves represent 4.46 u/t amplitudes. Intensity measurements and Fourier transform calculations have been described[2]. The models have side chains attached in the most probable conformation to the α-helix backbone[2]. The two strongest contributions of intensity in the 10 Å region are due to the periodicities of the 5-start and the 9-start helicoids, which are expressed in the helical Fourier transform as the J_5 and J_9 Bessel functions. The agreement between calculated and observed amplitudes over the whole diffraction pattern is similar to that of previous models[2]. The remaining differences between calculated and observed amplitudes are of the order usually found in fitting models to fibre diffraction data, and do not affect our discussion. *A*, Observed amplitude for the J_9 layer line. 4.40 u/t data for the layer line at $\zeta = 0.0128$ Å^{-1} (fibre AF 12/2, film 4055) and 4.46 u/t data for the layer line at $\zeta = 0.0046$ Å^{-1} (fibre AF 35/1, film 4158). *B*, Observed amplitude for the J_5 layer line. 4.40 u/t data for the layer line at $\zeta = 0.0383$ Å^{-1} and 4.46 u/t data for the layer line at $\zeta = 0.0278$ Å^{-1}. Film numbers as in *A*. The apparent discrepancy between the two curves beyond 0.1 Å^{-1} is due in part to the contribution of the J_{17} Bessel function, which cannot be resolved from J_5 in the 4.40 u/t data but is resolved (and not included) on the 4.46 u/t data. *C*, Calculated amplitude for the J_9 layer line (ζ as in *A*). *D*, Calculated amplitude for the J_5 layer line (ζ as in *B*).

way using a non-convergent energy minimisation technique which gave a local minimum for a series of models in the region of the transition[1].

Stereochemical constraints satisfactorily explain the limited nature of the transition, but they do not directly explain why the transition is driven by temperature. It is apparent that the stability of the 4.46 u/t helix relative to the stability of the 4.40 u/t helix changes with temperature. This effect could be due to temperature-dependent terms in the molecular interaction potentials controlling contact distances or patterns of attraction and repulsion between side chains. Changes in the interaction potentials could alter either the internal twist of the α-helix or the details of knobs-into-holes packing.

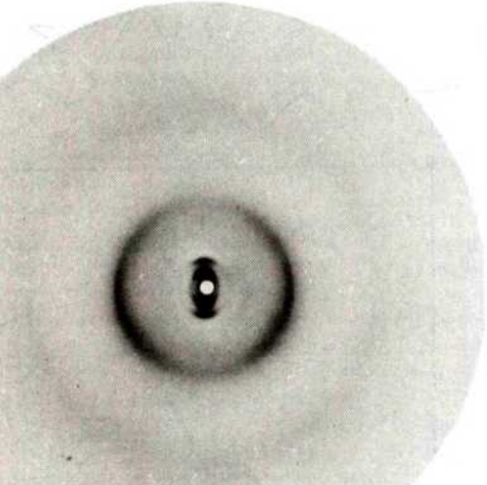

Fig. 4 X-ray diffraction pattern of ether-induced sheets of Pf1 bacterial virus. Plane of the sheet is horizontal. Sheets were prepared by shaking a purified solution of Pf1 at 5 mg ml^{-1} for 120 min at room temperature with an equal volume of ether[4]. The sheets were centrifuged at 30,000 r.p.m. for 3 h at 15 °C in the Beckman SW 50.1 rotor, and resuspended at a concentration of 25 mg ml^{-1} in 0.005M Tris-HCl, *p*H 8.1. A 0.5 ml drop of the concentrated solution was placed on a silicone-treated glass slide[23] and allowed to dry at 100% relative humidity, 4 °C, for 160 h in a 7-T magnetic field, with the plane of the slide perpendicular to the magnetic lines of force. A thin slice of the drop was cut free from the slide and mounted in the X-ray diffraction camera with the X-ray beam parallel to the plane defined by the slide. The sharp vertical reflections are at orders of 55.6 Å; on some patterns reflections up to the fifth order have been observed. The strong region of intensity in the horizontal direction is at 9 Å. Sample AF 33/2, film 4146, 75% relative humidity, specimen-film distance 7.4 cm, ×1.0. The same sample at 98% relative humidity expanded slightly to give sharp vertical reflections at orders of 60.1 Å.

Solvent is likely to play an important part in such phenomena. Even internal structure may be affected by solvent. Hydration behaviour can be subdivided[8] into ionic or electrostatic effects, hydrogen bonding of water to the solute, and hydrophobic effects. All of these are temperature dependent to some degree, and may play some part in the Pf1 transition. The electrostatic effect[9] is an especially likely candidate for the structural transition in Pf1. Theoretical calculations show that the dielectric constant of the solvent has an important effect on the conformation of proteins[10]. The dielectric constant of water increases as the temperature is reduced[11]. In the presence of glycerol (which has a dielectric constant about half that of water), the 4.40 u/t form is maintained even when fibres are made at 4 °C. This fact is consistent with the idea that the temperature-induced conformational change is due to the increase in the dielectric constant of the medium with decreasing temperature. Special cryosolvents[12] that have been developed to counteract the effect of temperature on *p*H may be useful to study the structural transition in Pf1.

It is possible that the system is instead controlled by entropy effects, resulting from vibration or libration of the protein molecules. Calorimetry could distinguish between the two possible mechanisms.

More dramatic changes in Pf1 structure can be induced by brief treatment with ether. The virions swell to hollow cylinders about three times their original diameter but only one tenth the normal length[13]; further treatment leads to flat sheets of protein[4]. X-ray diffraction studies of oriented stacked sheets show a strong 9 Å region of intensity in the direction parallel to the plane of the sheet, and sharp reflections at orders of about 55–60 Å in the direction perpendicular to the plane of the sheet (Fig. 4). This kind of diffraction pattern is expected if the Pf1 α-helical coat protein subunits are oriented with their long axes roughly perpendicular to the plane of the sheet. If the 46-residue α-helices were oriented exactly perpendicular to the sheets, the sheets would be 70 Å thick, but if some of the neighbour–neighbour side chain interactions in the sheet are the same as in the 5-start helicoids of the virion, the α-helices will be slightly skew and the sheets will be only 50–60 Å thick. The 9 Å spacing in the plane of the sheet corresponds to the distance between adjacent α-helices. Patches of coat protein in the bacterial membrane with the α-helical protein spanning the 50 Å thick membrane have been proposed as precursors of virus assembly[2,14–16]. These pure viral protein sheets are useful models for the study of assembly of the virion.

The Pf1 virion is an example of a system in which small local changes in molecular structure are amplified into large reversible changes in a molecular assembly. The changes within the α-helix subunit between the 4.40 u/t and the 4.46 u/t models are similar in magnitude to the calculated room temperature vibrations within an α-helix[17]. At the transition temperature, Pf1 will be delicately poised between the two structural forms, and little energy would be required to induce an oscillation between the two forms. This type of induced oscillation between two structural forms of an α-helical assembly could generate a travelling wave in other α-helical assemblies such as myosin or membrane pores[18,19].

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Local destabilisation of a DNA double helix by a T–T wobble pair

A. G. Cornelis, Jeroen H. J. Haasnoot, Jan F. den Hartog, M. de Rooij, Jacques H. van Boom & Altona Cornelis

Gorlaeus Laboratories, State University, PO Box 9502,
2300 RA Leiden, The Netherlands

Nuclear magnetic resonance is a technique which permits direct observation of the Watson–Crick hydrogen-bonded ring imino protons (guanine N_1H and thymine N_3H). As the formation and disruption of hydrogen bonds of double-helical RNA and DNA structures are key events during various biological processes, NMR thus provides a useful tool for studying the fluctuational mobility of the individual base pairs. Indeed, several NMR studies of oligo- and polynucleotides have been carried out to probe the structure and dynamics of nucleic acids in solution (for a review see ref. 1). The present study constitutes the first part of our attempt to assess the influence of non-complementary base pairs on the stability of nucleic acid double helices. We report the spectral assignment and temperature-dependent NMR profiles of the hydrogen-bonded imino protons of the two DNA fragments shown in Fig. 1. The assignment is based solely on experimental grounds using the principle of chemical modification. It will be demonstrated that the introduction of a non-complementary (wobble) base pair in a DNA duplex introduces an extra melting site in addition to the sequential melting which starts with the terminal base pairs in the double helix structure.

The oligodeoxynucleotides were synthesised using an improved phosphotriester approach[2]. Full details of preparation and purification will be published elsewhere[3]. Figure 2 presents the high-resolution 360 MHz ^{1}H-NMR spectrum between 12.0 and 14.5 p.p.m. of fragment 12 in H_2O as a function of temperature. Sequential linewidth and intensity changes with increasing temperature allow assignment of all signals to specific NH-Watson–Crick hydrogen-bonded protons[4]. At low temperatures (−3.5 °C) six resonances, two of which coincide at ~13.3 p.p.m., are observed. On raising the temperature the intensity of the signal at 13.3 p.p.m. falls from two to one proton, identifying that the faded signal as the fraying terminal A·T base pair 1. Between 10 and 30 °C the resonance at 13.8 p.p.m. gradually disappears and is therefore assigned to A·T base pair 2. The signal at 12.5 p.p.m. is next to follow (G·C base pair 3). At 52 °C only three signals remain. As G·C base pairs resonate at ~1 p.p.m. to higher field than corresponding A·T base pairs[5], the signal at 12.7 p.p.m. is assigned to the G·C base pair 4. Increasing the temperature to 56 °C results in the disappearance of all resonances indicating that the breaking of the last six base pairs is an 'all or none' process. The two A·T base pairs 5 and 6 can be assigned unambiguously by comparing the low temperature spectra of fragment 12 and fragment 13 (Fig. 3). In the spectrum of fragment 13 five out of six signals in the range of 12–14 p.p.m. display virtually the same chemical shifts as found in fragment 12; it is therefore concluded that the two fragments have similar conformations for the base pair sequence 1–5 and that the same assignment holds for fragment 13. The important difference, however, between the spectra of the two fragments is a large downfield shift of one of the resonances at 13.3 p.p.m. in fragment 12 to 14.3 p.p.m. in fragment 13. This shift is obviously due to the effect of separating base pairs 6 and 6′ by insertion of a T–T pair. The insertion moves the imino proton of base pair 6 away from the shielding cone of the A·T base pair 6′ and vice versa. Therefore, the resonance at 14.3 p.p.m. (and one of the resonances at 13.3 p.p.m. in fragment 12) is assigned to base pair

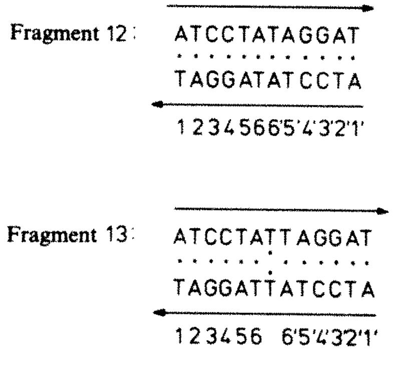

Fig. 1 Base sequence of the self-complementary deoxyoligonucleotides studied. Base pair numbering as shown; they are equivalent pairwise because of the two-fold symmetry of the double helix.

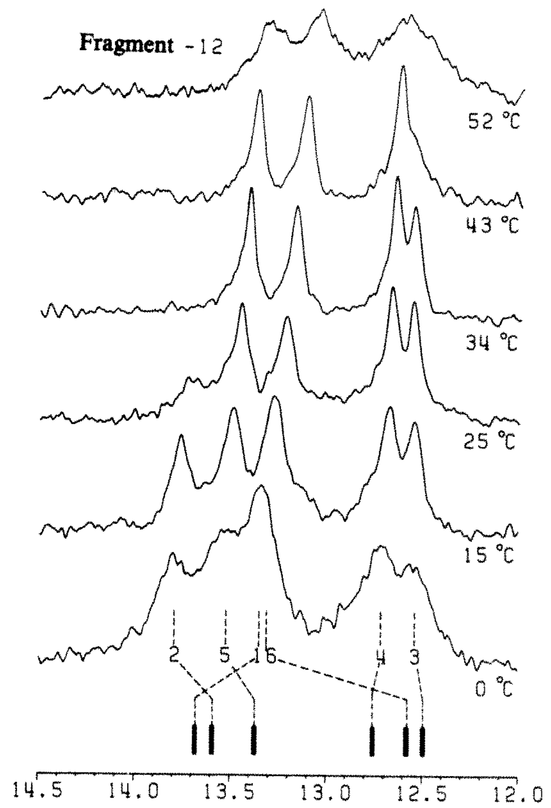

Fig. 2 Low-field region of the 360 MHz ^{1}H-NMR spectra of fragment 12 at various temperatures. Only a representative (6 out of 15) set of spectra is shown. The solid lines (bottom) represent the calculated shifts (see text) for the hydrogen-bonded imino protons in a B-DNA helix. The NMR samples were prepared by dissolving ~3 mg of the oligodeoxynucleotide in 270 µl of a buffer solution containing 0.5 M NaCl, 0.1 M sodium cacodylate (*p*H 7.0) and 5% v/v D_2O. High resolution proton NMR spectra were recorded on a Bruker HX-360 NMR spectrometer interfaced with a BNC-12 computer system and equipped with a standard temperature unit, using micro NMR sample tubes with a cylindrical cavity. The spectrometer was operated in the correlation mode[14], using an internal deuterium field/frequency lock. Chemical shifts were measured relative to the solvent H_2O peak and converted to DSS reference by correcting for the H_2O to DDS chemical shift using the appropriate temperature calibration curve. All reported shifts are denoted using the δ-convention, that is, positive values represent shifts to lower field.

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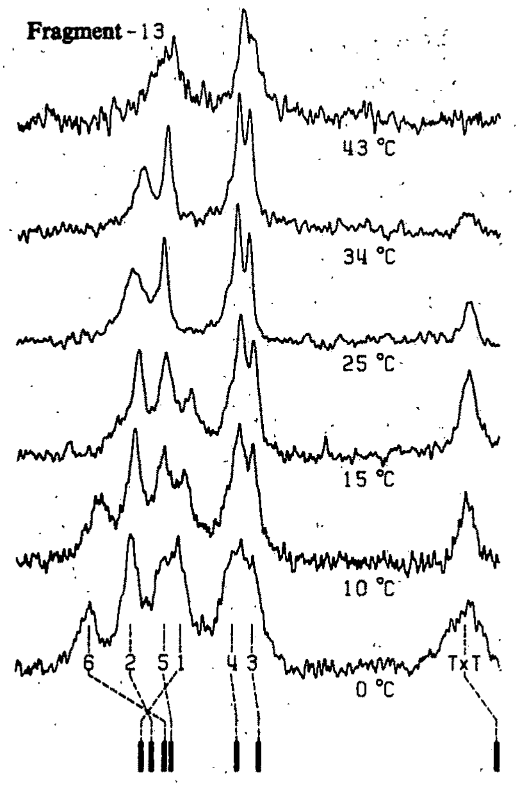

Fig. 3 Low-field region of the 360 MHz ^{1}H-NMR spectra of fragment 13 at representative temperatures. For further details see Fig. 2 legend.

6, and thus the remaining signal at 13.6 p.p.m. can be ascribed to base pair 5. An extra signal is displayed by fragment 13 at relatively high field (10.3 p.p.m.), which is assigned to the non-hydrogen-bonded imino protons of the thymidylyl residues in the middle of the strands. The temperature profile of fragment 13 is consistent with this assignment, but shows two 'melting sites' instead of one (see below).

It is now generally recognised that oligodeoxynucleotides tend to take up a B–DNA-like conformation in aqueous solution[6–8]. The computer program CARSHI (written in FORTRAN IV, using the parametrisation of Giessner–Prettre and Pullman[9]) was used to calculate the ring current shielding due to the nearest and next-nearest neighbouring base pairs in a B–DNA double-helical structure[10]. These values were added to the estimate chemical shift for 'isolated' Watson–Crick base pairs[5] ($\delta_{G-C}=13.6$ p.p.m.; $\delta_{A-T}=14.6$ p.p.m.), yielding the calculated shifts shown as solid lines in Figs 2 and 3. To determine the chemical shift of the 'free' (non-hydrogen-bonded) imino proton of a thymine residue the spectra of thymidine 5′-*O*-methyl phosphate and of d(TTTT) were recorded at 0 °C (~3 mM, *p*H = 7). Both compounds displayed a very broad resonance at 11.2 p.p.m., which sharpened when the exchange rate was suppressed by lowering either the temperature (to −9 °C) or the *p*H (to 4.0).

The agreement between calculated and observed chemical shifts seems reasonable, taking into account the possible and unknown effects of residual fraying and/or small deviations from the accepted B–DNA structure. However, it is clear that assignment of resonances close to one another on the basis of ring current shift calculations must be regarded with caution.

Fragment 12 represents the first oligodeoxynucleotide studied by nuclear magnetic resonance in which A·T base pairs in the middle of the strands are flanked on both sides by G·C base pairs. A·T-rich regions are known[11] to be thermally less stable than G·C-rich regions in long DNA fragments. One could therefore expect that the central A·T region in fragment 12 would 'melt out' before the G·C pairs disappeared. This is not the case; our experiments clearly indicate that all four central A·T base pairs in fragment 12 remain intact until the last G·C base pairs 4 and 4′ are disrupted. Therefore, we conclude that a 'G·C protected' sequence of four A·T pairs is too short for such a local destabilisation of the duplex.

For fragment 13, it is certainly important to determine whether the T–T pair loops out from the helix or whether it forms a wobble pair. The present experiments give the following pertinent information: the chemical shift of the T–T imino protons are ~1 p.p.m. to higher field than that observed for the 'free' imino proton of a thymine residue (see above). This large value for the upfield shift requires that this 'free' imino proton is positioned in the shielding cone of the neighbouring adenine base. Therefore, we propose that fragment 13 has a marked tendency to form a continuous, regular B-DNA-like structure with a T–T wobble pair embedded in the middle of the helix. Shift calculations agree well with this proposition (Fig. 3). Interestingly, a similar occurrence was recently reported in a temperature-jump study[12] of anticodon–anticodon interactions between various tRNAs. It was found that the U–U wobble in the middle position of the interacting codons behaves in a unique way in the sense that it apparently does not oppose the formation of stable complexes, whereas the other mismatches tested (C–C, A–A, G–G, G–A, C–A) seem to prohibit complex formation. Thus, the continuity of the DNA double helix with a T–T wobble pair may be found to be an exception rather than a rule, but further work is necessary to clarify this point.

The temperature profile of fragment 13 displays several interesting features. Compared with fragment 12, the NMR melting temperature is lowered by ~11 °C, indicating that the insertion of the T–T wobble pair significantly lowers the overall stability of the double helix. This observation agrees with thermal elution studies of oligodeoxyadenylates on cellulose-pdT$_9$. The replacement of an internal dA- by a dT-residue lowered the elution temperature (which is compatible with the T_m obtained from solution thermal denaturation studies) by about 15 °C (ref. 13). An explanation of this phenomenon may be inferred from Fig. 3. The resonances of the T–T wobble pair and A·T base pair 6 located in the middle of the double-stranded structure vanish within the same temperature range as the terminal A·T base-pair 1. This implies that the wobble introduces an extra melting site in the double-helical structure. Thus, the breaking of the Watson–Crick hydrogen bonds occurs on both sides of the two G·C pairs, causing fragment 13 to be destabilised relative to fragment 12.

This study clearly demonstrates that high-field nuclear magnetic resonance techniques combined with 'tailor-made' oligonucleotides significantly increases our understanding of the structural features and dynamics of this type of important biological model compound.

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Thromboxane molecules do not adopt the prostaglandin hairpin conformation

D. A. Langs, S. Fortier, M. G. Erman & G. T. DeTitta

Medical Foundation of Buffalo, Inc., 73 High Street, Buffalo,
New York 14203

The hairpin conformational hypothesis[1] has been proposed to rationalise much of the structure–activity and receptor-binding data which have accumulated for the prostaglandin (PG) hormones. The hairpin conformation, thought to be necessary for PG activity, requires that the α- and ω-chains of the molecule be extended and in parallel alignment, separated by a van der Waals contact distance for the full length of the chains, with the ends of the chains approximately 5.5 Å apart. The similarity between the structures of the thromboxanes (TXs) and the PGs suggests that the profile of activity of TXs, like that of PGs, centres on subtle conformational variation of the hairpin geometry. Thromboxane B_2 (TXB_2) is a stable hydrolysis product[2] of a highly reactive, short-lived intermediate[3], thromboxane A_2 (TXA_2), which is formed from the prostaglandin endoperoxide (PGH_2) as indicated in Fig. 1. An examination of molecular models of TXA_2 and TXB_2 suggests that the structural differences between the ring moieties may have much less influence in altering the side-chain conformations of TXs than do substituents on the relatively more flexible cyclopentane ring of a PG molecule. We report here the first diffraction analysis of a thromboxane structure and note that the molecular conformation is not hairpin shaped.

Thromboxane B_2 was crystallised from ethyl acetate as colourless, transparent, monoclinic laths following suggestions offered by Hanessian[4]. The space group is $P2_1$, with unit cell data $a = 9.971(3)$, $b = 40.45(2)$, $c = 5.510(2)$ Å, $\beta = 102.5(1)°$, $Z = 4$, D_{obs} (flotation) $= 1.141 \text{ g cm}^{-3}$, $D_{calc} = 1.138 \text{ g cm}^{-3}$. There are two crystallographically independent TXB_2 molecules in the unit cell. The diffraction intensities were recorded from a prismatic specimen ($0.12 \times 0.12 \times 0.40 \text{ mm}^3$) in the θ–2θ scan mode on an automated diffractometer fitted with a CuKα source. A total of 2,272 independent reflections were measured out to a 2θ angle of 100°. Of these, only 1,227 intensities were considered observable ($I \geq 2\sigma I$). The crystal structure was determined by direct methods and refined isotropically by full matrix least-squares procedures to the current residual $R = 0.145$. The general weakness of the diffraction data is consistent with the high thermal motion observed for the terminal carbon atoms of the ω-chains and the disorder in $O_{(11)}$ (α and β epimers) for one of the molecules in the structure.

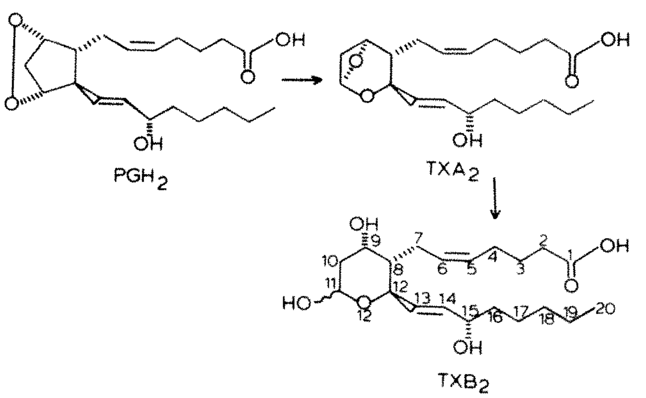

Fig. 1 Chemical formulae and numbering scheme for TXB_2 and its immediate precursors.

The two crystallographically independent molecules and their overlapped composite are shown in Fig. 2. The composite illustration was derived by overlapping corresponding atoms of both molecules in the least-squares sense[5] for the common contiguous 12-atom moiety from $C_{(6)}$ to $C_{(15)}$, excluding $O_{(11)}$. Note the torsional dissimilarities at the $C_{(4)}$–$C_{(5)}$ and $C_{(6)}$–$C_{(7)}$ bonds (Table 1). The carboxyl side chains bend sharply towards either the α or β face of the molecule. For convenience, we refer to these as the α-tail and the β-tail 'scorpion' conformers, respectively. The TXB_2 ω-chain conformations are similar but distinct. The $C_{(16)}$–$C_{(17)}$ bonds are both +synclinal rather than transplanar, as noted for every one of 12 PG ω-chain conformations studied by diffraction methods. The kinking of the chain at the $C_{(16)}$–$C_{(17)}$ bond has the effect of foreshortening the ω-chain such that it extends only as far as $C_{(4)}$ for its terminal contact with the α-chain. It can be seen from the molecular overlap figure that the $C_{(13)}$–$C_{(14)}$–$C_{(15)}$–$C_{(16)}$ torsion angles, which define the orientations of the tails of the ω-chains and which differ by 16 °, cause these tails to be slightly shifted either up or down to achieve this terminal contact with $C_{(4)}$. The end of the carboxyl chain is not stabilised by van der Waals association with the ω-chain as is the case in the hairpin conformation, and may be conformationally flexible with regard to the $C_{(4)}$–$C_{(5)}$ hinge point. Although molecular models might suggest that viable hairpin conformers of TXs may be formed close to the mean plane of the pyranose ring, we consider that such hairpin models generally crowd the side chains more closely together than if they had emanated from a cyclopentane head group. A +anticlinal $C_{(6)}$–$C_{(7)}$ bond conformation has previously been observed for each of four conformers of $PGF_{2\alpha}$ (refs 6, 7). The

Table 1 Thromboxane B_2 torsion angles for β-tail and α-tail conformers

$O_{(1A)}$	$C_{(1)}$	$C_{(2)}$	$C_{(3)}$	2(10)
$C_{(1)}$	$C_{(2)}$	$C_{(3)}$	$C_{(4)}$	−177(176)
$C_{(3)}$	$C_{(4)}$	$C_{(5)}$	$C_{(6)}$	−142(144)
$C_{(5)}$	$C_{(6)}$	$C_{(7)}$	$C_{(8)}$	91(−148)
$C_{(6)}$	$C_{(7)}$	$C_{(8)}$	$C_{(12)}$	173(−177)
$C_{(7)}$	$C_{(8)}$	$C_{(9)}$	$C_{(10)}$	176(175)
$C_{(7)}$	$C_{(8)}$	$C_{(12)}$	$O_{(12)}$	176(180)
$C_{(8)}$	$C_{(12)}$	$C_{(13)}$	$C_{(14)}$	−144(−123)
$C_{(9)}$	$C_{(10)}$	$C_{(11)}$	$O_{(11B)}$	168(−172)
$C_{(9)}$	$C_{(10)}$	$C_{(11)}$	$O_{(12)}$	49(50)
$C_{(9)}$	$C_{(8)}$	$C_{(12)}$	$O_{(12)}$	−55(−51)
$O_{(9)}$	$C_{(9)}$	$C_{(8)}$	$C_{(12)}$	−70(−71)
$C_{(10)}$	$C_{(11)}$	$O_{(12)}$	$C_{(12)}$	−62(−59)
$O_{(11B)}$	$C_{(11)}$	$O_{(12)}$	$C_{(12)}$	179(161)
$C_{(12)}$	$C_{(13)}$	$C_{(14)}$	$C_{(15)}$	180(180)
$C_{(13)}$	$C_{(14)}$	$C_{(15)}$	$C_{(16)}$	−118(−134)
$C_{(14)}$	$C_{(15)}$	$C_{(16)}$	$C_{(17)}$	56(60)
$O_{(15)}$	$C_{(15)}$	$C_{(16)}$	$C_{(17)}$	177(−177)
$C_{(17)}$	$C_{(18)}$	$C_{(19)}$	$C_{(20)}$	174(−55)
$O_{(1B)}$	$C_{(1)}$	$C_{(2)}$	$C_{(3)}$	172(179)
$C_{(2)}$	$C_{(3)}$	$C_{(4)}$	$C_{(5)}$	177(174)
$C_{(4)}$	$C_{(5)}$	$C_{(6)}$	$C_{(7)}$	1(−14)
$C_{(6)}$	$C_{(7)}$	$C_{(8)}$	$C_{(9)}$	44(58)
$C_{(7)}$	$C_{(8)}$	$C_{(9)}$	$O_{(9)}$	61(56)
$C_{(7)}$	$C_{(8)}$	$C_{(12)}$	$C_{(13)}$	55(60)
$C_{(8)}$	$C_{(9)}$	$C_{(10)}$	$C_{(11)}$	−42(−49)
$C_{(8)}$	$C_{(12)}$	$O_{(12)}$	$C_{(11)}$	64(61)
$C_{(9)}$	$C_{(10)}$	$C_{(11)}$	$O_{(11A)}$	(−87)
$C_{(9)}$	$C_{(8)}$	$C_{(12)}$	$C_{(13)}$	−176(−172)
$O_{(9)}$	$C_{(9)}$	$C_{(10)}$	$C_{(11)}$	77(69)
$C_{(10)}$	$C_{(9)}$	$C_{(8)}$	$C_{(12)}$	47(48)
$C_{(11)}$	$O_{(12)}$	$C_{(12)}$	$C_{(13)}$	−166(−178)
$O_{(11A)}$	$C_{(11)}$	$O_{(12)}$	$C_{(12)}$	(68)
$O_{(12)}$	$C_{(12)}$	$C_{(13)}$	$C_{(14)}$	122(117)
$C_{(13)}$	$C_{(14)}$	$C_{(15)}$	$O_{(15)}$	120(110)
$C_{(15)}$	$C_{(16)}$	$C_{(17)}$	$C_{(18)}$	58(71)
$C_{(16)}$	$C_{(17)}$	$C_{(18)}$	$C_{(19)}$	166(175)

Torsion angles are given to the nearest whole degree. Estimated standard errors in these values range from 2.5 to 7.0° at the C_{20} chain ends. The β-tail values are given first, followed by the α-tail values in parentheses.

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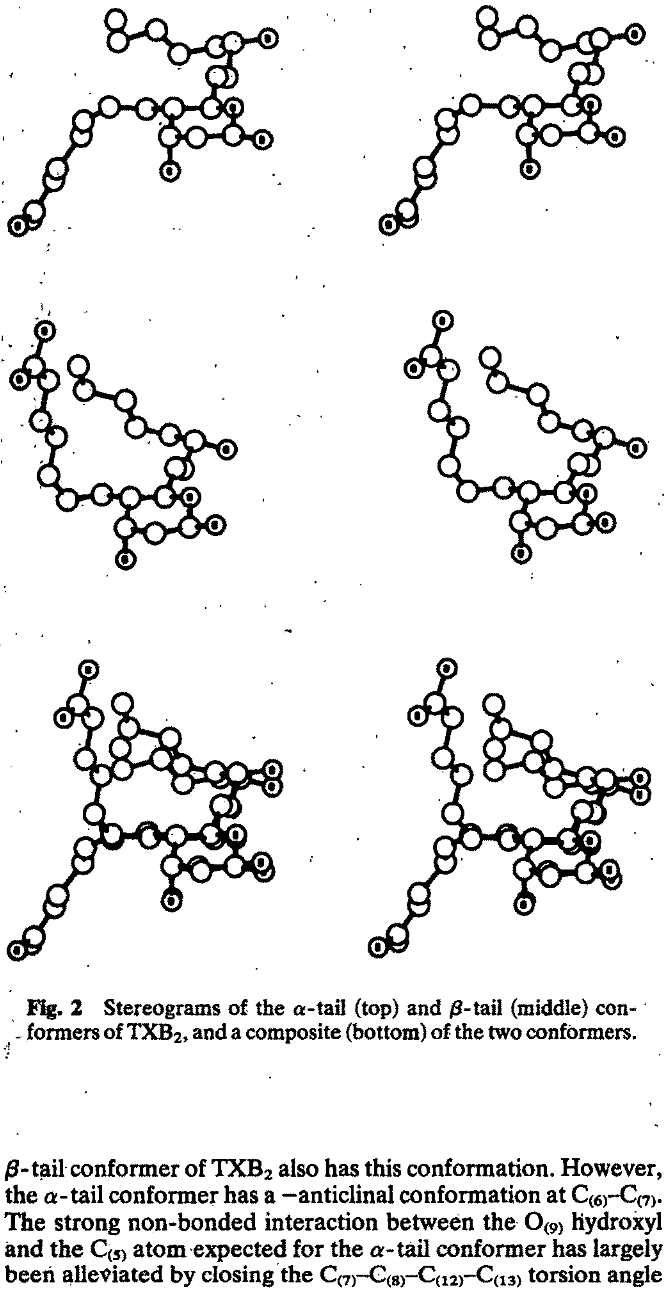

Fig. 2 Stereograms of the α-tail (top) and β-tail (middle) conformers of TXB_2, and a composite (bottom) of the two conformers.

β-tail conformer of TXB_2 also has this conformation. However, the α-tail conformer has a −anticlinal conformation at $C_{(6)}$–$C_{(7)}$. The strong non-bonded interaction between the $O_{(9)}$ hydroxyl and the $C_{(5)}$ atom expected for the α-tail conformer has largely been alleviated by closing the $C_{(7)}$–$C_{(8)}$–$C_{(12)}$–$C_{(13)}$ torsion angle from the average value of $+86 \pm 5°$ noted for the $PGF_{2\alpha}$ structures to the observed average of $+58 \pm 4°$ determined for the pyranose side-chain ring junctions of TXB_2.

Whereas the β-tail conformer is identified solely as the 11-β epimer, the α-tail conformer seems to be approximately 65% 11-β epimer and 35% 11-α epimer. Although this corresponds roughly to the 2:1 ratio noted for the unspecified methoxy-TXB_2 epimers resulting from the TXA_2 trapping experiments[3], these latter values relate to a different transition state equilibrium from that associated with anomeric equilibrium processes. The two conformers associate as carboxylic acid dimers in the crystal structure.

TXB_2 is a moderately bronchoactive metabolite[8] which survives degradation by 15-hydroxy-prostaglandin dehydrogenase (PGDH) in the lung[9,10]. The $C_{(15)}$ hydrogen positions of both conformers may be obstructed from the β-facial approach[11,12] of the nicotinamide group of NAD^+ by the +gauche twist in the ω-chain at $C_{(16)}$–$C_{(17)}$; in addition, the $C_{(15)}$ hydrogen may be obstructed by the α-chain in the β-tail conformer. This may constitute a desirable but not necessarily essential condition for TXB_2 resistance to PGDH metabolism, as the scorpion conformations may be sufficiently different to prevent binding. Although significant amounts of TXB_2 are degraded by PGDH in an antigen-challenged isolated perfusion preparation of sensitised guinea pig lung tissue[13], this is not at variance with its resistance in normal physiological conditions. This observation does suggest, however, that thermodynamic preference for the scorpion conformers is not sufficient to prevent less probable but conformationally more compatible forms of the substrate from reacting with the enzyme over prolonged exposure periods.

TXA_2 is physiologically active as a constrictor of coronary arteries[14] and as a potent platelet aggregator[3] in thrombus formation, whereas TXA_1, derived from PGH_1, lacks both activities. This has implied the chemical importance of *cis*-Δ^5 unsaturation for hormonal recognition in platelets and vascular smooth muscle[15,16]. The present study calls attention to the conformational importance of the *cis*-Δ^5 bond to produce α-tail and β-tail scorpion conformers. It remains to be shown whether the structure of TX receptors fits either of these conformational models and whether the conformational requirements for platelet and smooth muscle receptors are different.

The TXB_2 samples used in this study were synthesised[17–19] at the Upjohn Company, and provided by Drs Bundy and Pike. This work was supported by grant no. HL-15378 awarded by the National Heart, Lung and Blood Institute, DHEW.

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Corrigendum

In the article 'Radio studies of the double QSO, 0957+561A,B' by G. G. Pooley *et al. Nature* **280,** 461–464, in Table 2 the entry for radio component A should indicate a size of 1 arc s. In the equation below Fig. 3, for . . . independent of data . . ., read . . . independent of β.

Erratum

In the article 'Coden–anticoden interaction at the ribosomal peptidyl site' by R. Lührmann *et al. Nature* **280,** 423–424, Figures 1 and 2 are transposed. Figure 1 shows dependence on $[Mg^{2+}]$.

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reviews

Tour of statistical history

Stephen M. Stigler

The History of Statistics in the Seventeenth and Eighteenth Centuries, against the changing background of intellectual, scientific and religious thought. Lectures by Karl Pearson given at University College, London, during the academic sessions 1921-1933. Edited by E. S. Pearson. Pp. 744. (Charles Griffin: High Wycombe, UK, 1979.) Paperback £7.50.

Pearson with the Brunsviga adding machine, 1910

Courtesy University College, London, UK

KARL PEARSON combined apparently boundless energy with a wide-ranging enquiring intelligence and indomitable perseverance to produce a remarkable career as a philosopher, physicist, mathematician, statistician, historian, editor, biologist and teacher. Pearson will doubtless be best remembered as a statistician. His first statistical publication was a letter to *Nature* on asymetrical frequency curves (published October 26, 1893). Over the succeeding decade he produced such widely known techniques as the χ^2 test, and he was primarily responsible for the early development of correlational analysis. The fact that such a basic term as "standard deviation" was first introduced by Pearson is testimony to the ubiquity of his influence upon subsequent statistical thought. When Pearson died in 1936, at the age of 79, he left behind a published legacy so extensive that a medium-sized hardcover book was required for his bibliography alone. He also left behind a large archive of correspondence and unpublished lecture notes, and it is from this latter source that this present volume has been drawn.

Before Pearson's emergence as a researcher in statistical methods in 1893, he had demonstrated an interest and ability in the history of science through his editing and completion of the second volume of Isaac Todhunter's *A History of the Theory of Elasticity and of the Strength of Materials from Galilei to the Present Time* (1893). After the War his thoughts returned to historical matters, as he sought to complete a massive biography of Francis Galton, and from 1921 to 1933 he gave a course of lectures at University College, London, on the history of statistics. Fortunately for all of us the notes of these lectures have survived and found the perfect editor — Pearson's son Egon S. Pearson, himself justly celebrated for both his innovative research in statistics and his insightful accounts of its history.

Karl Pearson was known as a spell-binding lecturer, and a good sense of his pace, method and vocal energy comes across on these pages. Apparently Pearson's habit was to draft his lectures *in extenso,* as if for reading, and it is these drafts which have survived. Thus, the present work is essentially a verbatim recording of the lectures of a master lecturer, a connected and detailed account of its topic at a pace (and with the necessary digressions) to hold a listener's attention. For the most part this is not the dry chronological account that so many histories of this field become, but it is Karl Pearson speaking to us. Sometimes he is earnest and scolding, as when he remonstrates with d'Alembert for a failure to test his philosophical views experimentally (page 542); sometimes he goes off on interesting, if irrelevant digressions, as when he disputes statements that Lagrange's first marriage had been incompatible (page 578). Occasionally there are flashes of humour, as when, after explaining how Lagrange loved to abandon himself to mathematical thought while listening to music, Pearson wrote: "Luckily 'Jazz' was not then invented, or the result might have made him a circle-squarer, rather than one of the greatest of mathematicians" (page 576).

These lectures cover the period from John Graunt's 1662 *Observations on the London Bills of Mortality* to Laplace's 1812 *Théorie Analytique des Probabilités,* with considerable emphasis on biographical detail and personality. The coverage reflects Pearson's interests and his taste for philosophy. There is extensive discussion of early work by the political arithmeticians (including estimation of population, construction of life tables, and inference about the sex ratio), but very little on the handling of astronomical or geodetical data. Pearson took a particular fancy to individuals with an interest in both mathematics and theology, and Richard Price receives the benefit of 52 pages of discussion. In his discussion of the later mathematicians, Lagrange and Laplace, Pearson's attention is more focussed on mathematical detail, in the manner of Todhunter's *A History of the Mathematical Theory of Probability.* But even here he exults when he encounters an antecedent to one of his own results. His discovery (page 599 *et seq.)* that Lagrange had almost derived the χ^2 distribution in about 1770 shows us both the excellence of Lagrange's mathematical skills and Pearson's own tendency to elevate mathematical form above statistical concept.

As a history of statistics, the book is not without its defects, and many are pointed out by the editor in his preface. There are errors of fact (though few misprints) that Pearson would likely have caught had he revised the lectures himself, and at times the length of the exposition makes for heavy going. The views presented of past accomplishments are dated by being rooted in Pearson's own work, and the background of religious thought and political movement that

Pearson gives in many extensive digressions is not, I think, successfully integrated into the discussion of the development of statistical methods. Bibliographical details are generally given accurately, but references to even those secondary sources available to Pearson are scarce. But these are all minor problems compared with the rare opportunity we have been given to sit at the feet of this energetic and learned professor as he takes us on a personalised tour of statistical history, and we often learn as much in the process about Pearson as we do of his subject. Here Pearson speaks of a table of Simpson's as "probably faked" (page 387); there Pearson laments that the brains of Lagrange and Laplace were not preserved for later study (page 585); and there Pearson faults Euler for failure to compile numerical tables "which would energise the symbols" (page 245). Whether we are annoyed by his opinionated view of history or enthralled by his enthusiasm, we cannot fail to be fascinated and grateful for this chance to witness these lectures.

The editor, E. S. Pearson, has done an excellent job of piecing together the different versions of several lectures, furnishing a useful index and many notes, and cutting wisely where cutting was needed. The publishers must be congratulated for keeping the price at what must, for a volume of this size, be considered low. We may hope that this encourages many scientists (not only statisticians) to make the acquaintance of the extraordinary Karl Pearson. □

Stephen M. Stigler is a Professor in the Department of Statistics at the University of Chicago, Illinois.

Nutrition planning

Nutrition and National Policy. Edited by Beverley Winikoff. Pp. 580. (MIT Press: Cambridge, Massachusetts, and London, UK, 1979.) £15.75.

THIS book is a monument to nutrition planning, the latest enthusiasm to sweep the nutrition profession as it looks desperately for some effective action with which to improve the health problems of developing countries.

Nutrition planning is a controversial approach the more coherent advocates of which argue that protein energy malnutrition in children must not be seen simply as a disease entity demanding clinical treatment. It is a disease of the poorest of the poor, a symptom of deprivation, the product of a lack of wealth and welfare which can only be eliminated by action at the economic level. As the object of development planning is to help reduce these same inequalities, three important consequences follow. Nutritional status will be improved by successful development strategies, and the improvement in nutritional status must be one of the objectives of a good development policy. Conversely, the extent of malnutrition serves as an indicator of the effectiveness of those programmes.

This approach is one that I and many of my colleagues find acceptable. Many others do not agree, finding it unrealistic or unnecessary. These are legitimate differences in opinion, a source of mutual respect and a fruitful academic dialogue.

Unfortunately, although nutrition planning is an exciting theory, it is also a popular idea. The concept of nutrition planning has not been accepted but the image has, and this has become the portmanteau justification of tarted up versions of the same tired old theories that failed in the past. Sadly, ideas change slowly: they are simply renamed and thereby extended.

From all this comes, I assume, this book — almost 600 pages of mostly unnecessary prose with all too few passages that are worth reading. Two-thirds of the book are devoted to 'case studies' of national nutrition policies largely by the representatives of various developing countries. Apart from a brilliant study of malnutrition in Chile by G. Solimano and P. Hakim, which is a précis of their book (*Development, Reform, and Malnutrition in Chile*, MIT Press; for review, see *Nature*, **276**, 542; 1978) on this topic, these national presentations are mediocre indeed. If nutrition students presented, as their view on nutrition planning, the hotch potch of miscellaneous data that seem to satisfy some of these planners they would fail their degrees. Why then are they included here? One possible reason lies in the origins of the book, which is the edited proceedings of a conference funded by the Rockefeller Foundation which took place at a Rockefeller mansion in Bellagio, Italy in 1975.

There is a growing tendency in international jamborees devoted to such areas of nutrition to invite participants not by eminence, or relevance, but by race. This may be politically expedient, but it is bad for science, and ultimately insulting to those who only get invited as statutory representatives of the third world. I do not know how far this consideration governed the structuring of this meeting, but, distasteful though the idea is, it would explain the quality of the papers selected, which add little to human understanding and detract from the science of nutrition.

It seems to me that nutritionists in government should at least have details of the prevalence of malnutrition in their countries, and changes in that rate with development policy. No such luck. If these authors are really reporting the understanding of nutrition planners in their countries, then either systematic data is not being collected, or no-one exploits it in the way that is surely a precursor to its use in the political decision making process.

We do not need them to report over and over again that malnutrition occurs in children, or even that the poor are nutritionally worse off than the rich. Such information is not in doubt. It is clear that the poor suffer. What is also clear is that even at the worst not all the poor are malnourished. Nutrition planning will only proceed effectively when the political decision makers are given some idea of which socially or ecologically defineable groups are at greatest risk, so that social policy can then be designed to help those groups.

In this book economic and nutritional information is presented in abundance, but it is presented neither within a framework that presupposes such action nor in the form that can be used by the reader to develop his own theories. I cannot imagine a politician being interested in such ramblings. These are not case studies they are travelogues compounded of largely uncollated nutritional and economic data, and peppered with politically tactful remarks.

When the book gets on to generalisations it improves somewhat, but still says little at great length. Towards the end it is suggested that Hong Kong, Singapore, South Korea and Japan have radidly improved nutritional status as a result of income redistribution. I am not convinced, but given that it is even tenable why doesn't the Rockefeller Foundation now fund a less lavish session to discuss the situation in these countries. If it were done coherently, those of us interested in this proposition could see whether given the political will, economics alone was the answer to nutritional problems. That at least would provide a test of the nutrition planning hypothesis in a way that the present volume notably fails to do.

John Rivers

John Rivers is Lecturer in the Department of Human Nutrition at the London School of Hygeine and Tropical Medicine, London, UK.

- In the review of *Atomic Absorption Spectroscopy* (see *Nature*, **280**, 705; 1979), the author's name was incorrectly quoted. This should have read M. Slavin.
- *Origins: What New Discoveries Reveal about the Emergence of Our Species and Its Possible Future* (for review, see *Nature*, **270**, 108; 1977), by Richard Leakey and Roger Lewin, has been published in paperback by Macdonald and Jane's (London, UK).

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Atlas of economic mineral deposits

Atlas of Economic Mineral Deposits. By C.J. Dixon. Pp. 143. (Chapman and Hall: London, 1979.) £35.

MINERAL deposits of economic grade represent in all cases abnormal concentrations of the contained elements resulting from unusual combinations of geological processes. This is equally true whether the deposits are of metalliferous ore or industrial minerals, and such deposits are dispersed very thinly through the outer crusts of the Earth. Their bulk is usually small compared with that of the rocks with which they are associated. These fundamental points are implied in Colin Dixon's original and interesting *Atlas*, but they might have received more specific emphasis. His book deals with solid, non-combustible mineral deposits, and bears upon energy sources only in the case of uranium minerals. The deposits with which he is concerned supply, however, most of the raw materials for human artefacts, the manufacture of which is the purpose of the greater part of industry. The *Atlas* is intended as a reference work and a source of descriptive material to accompany a university course. The student of geology whose course includes (regrettably!) no discussion of the useful minerals would also find this a good text to read.

In nine pages of introduction set in three-column format Dixon deals with the generalities of the subject, including prospecting, exploitation, beneficiation and, briefly, the economics of the business; also the scientific aspects including petrology, morphology, and distribution, concluding with a neat summary of the history of genetic interpretation. Some geologists might have been tempted to generalise more about the structural control of oreshoots — the area where the application of geological reasoning has contributed most to industry — but this emerges well enough from the detailed descriptive pages that follow. Here, instead of attempting a comprehensive survey, forty representative major mineral-producing districts are illustrated and summarised, both aspects being ably presented. A very brief list of references accompanies each description forming a tailpiece to the accounts, each of which occupies a double folio of the 240 x 400 mm format. In most cases the drawings, on which a uniform system of symbols is used, are simplified from the published originals, often with great advantage in bringing out the essential points under discussion. Almost all teachers of the subject would agree with the author's contention that the subject needs visual presentation.

The major grouping is fourfold: (1) deposits in environments at the Earth's surface; (2) in sedimentary rocks; (3) associted with felsic magmatic environments; (4) in basic and ultrabasic magmatic rocks. This implies only a loose genetic classification, and wisely so, as it avoids the still-controversial question as to whether the mineral concentrations in sediments were formed in the normal course of sedimentation or not. As the author remarks, a group of French and other continental workers feel strongly that in fact they are so formed, but I am glad that he was not able to think of an English translation of *la gîtologie* for this term seems to me to be very closely associated with this school, the conclusions of which many of us find quite unacceptable in the light, for example, of inclusion homogenisation and stable isotope studies. The compromise proposal which says that minerals concentrated during some later diagenetic episode are sedimentary in origin also seems unsatisfactory to some of us. Mr Dixon wisely avoids confrontation by saying that the terms syngenetic and epigenetic are difficult to apply. His grouping brings together the exogenous deposits in (1) and it is open to those who wish to do so to think that the deposits in groups (2) to (4) derived their energy of formation from processes internal to the Earth's crust and mantle.

The forty examples selected give a good picture of the range of different geological environments in which valuable deposits may become concentrated. The processes involved probably varied as widely as the environments in which they operated. If there is any common factor, surely it is the efficacy of brine as a solvent of metals and other useful elements, whether as sea-water passing through hot basic lavas extruded on the ocean floor, or connate fluid sucked from sediments through cooling felsic magmas, or hypersalines forced out of deep consolidating sedimentary basins. But no single mechanism will cover all possibilities and we should agree with the author that the multiple hypothesis remains unavoidable.

A final section contains five world-distribution maps, and appendices on mineral names, units of measurement and stratigraphic nomenclature. But for the price, I would recommend every student of geology to acquire this book. The hope should be expressed that the unusual shape will not lead to it becoming hidden in obscure corners of college libraries.

Kingsley Dunham

Sir Kingsley Dunham was Director of the Institute of Geological Sciences from 1967-75.

Digging into Wittgenstein

Ludwig Wittgenstein: Remarks on the Foundations of Mathematics. Revised edition. Edited by G.H. von Wright, R. Rhees and G.E.M. Anscombe. Translated by G.E.M. Anscombe. (MIT Press: Cambridge, Massachusetts, and London, 1978.) $27.50.

THIS is a considerably expanded version, in English only, of the book first published under the same title, in English and German, in 1956. It consists, as the editors explain in their Preface, of selections from Wittgenstein's manuscript writings on the philosophy of mathematics from 1937 to 1944, together with nine pages written in 1933-34. It is divided into seven parts, with three appendices to Part I, each part being taken from a different manuscript; but, as the editors make clear, only Parts I and VI reproduce Wittgenstein's text *in toto*, the others constituting selections made by the editors from more extensive writings. The editors express "the opinion that the time has not yet come to print the whole of Wittgenstein's MSS on these and other topics", but give no reasons.

It is now approaching thirty years since Wittgenstein's death; and it is only quite recently that what might be called the philosophical community has come to real grips with the most difficult and profound of his ideas. In that time, much chaff has blown away. The heady excitement of logical positivism, and, after it, of Oxford ordinary language philosophy, can be looked back on only with some embarrassment as intoxication with crudely over-simplified ideas. Wittgenstein's work remains; undeniably, now, that of one of those few philosophers who will be read by all future generations. It is by far the richest twentieth-century source of philosophical ideas, which it will take us more decades yet properly to apprehend and to absorb; despite the difficulty with which his work presents the reader, there is nothing that is likely to be more rewarding. The philosophy of mathematics was one of his earliest and most persistent preoccupations. Originally he planned to include a lengthy discussion of it in the *Philosophical Investigations*, but, being dissatisfied with what he had written, cut almost all of it out; this material comprises Part I of the present book. He was evidently never satisfied with the work he had done in this area of philosophy, and, as the editors remark, did not return to it after April, 1944. The present edition is thus in no sense a finished treatment, or even a unitary book, any more than was the original one: it is a selection from seven distinct pieces of writing by Wittgenstein, with none of which he was content. For all that, it demands the most thorough attention from anyone interested in his philosophy, because the subject occupied so important a place in his thought. He obviously believed that his basic philosophical ideas had profound consequences for the philosophy of mathematics. Precisely what he took these consequences to be is something that the reader has to work very hard to discover.

Wittgenstein's mode of composition was unique. He filled notebooks with 'remarks', a few lines in length, which he continually revised; he would also constantly rearrange the 'remarks' in different sequences to form a disjointedly continuous text. Sometimes the import of the individual remark is luminously clear, sometimes quite obscure: but the method of composition obviously risks leaving the sequence of thought difficult to follow. That it need not have this effect is apparent from the *Investigations*, the only product of his later period that Wittgenstein regarded as a (nearly) finished book: there the final product of years of revision and rearrangement provides a largely continuous treatment of a range of topics, and, given close concentration on the reader's part, expresses with considerable clarity Wittgenstein's conclusions concerning them. Unfortunately, such an outcome to so strange a mode of composition was usually attained only at the final stage: the present book contains seven different sequences of remarks, five of them with omissions made by the editors, and none of them representing a final stage. It is difficult to convey the obstacles which this presents to comprehension without extensive quotation, but perhaps the following brief sequence from Part IV may be regarded as not untypical:

> 37. A human being is *imprisoned* in a room, if the door is unlocked but opens inwards; he, however, never gets the idea of *pulling* instead of pushing against it.
>
> 38. When white turns black some people say "Essentially it is still the same"; and others, when the colour turns a shade darker: "It is *completely* different".
>
> 39. The proposition '$a = a$', 'p p' ['If p, then p'], 'The word "Bismark" has 8 letters', 'There is no such thing as reddish-green', are all obvious and are propositions about essence: what have they in common? They are evidently each of a different kind and differently used. The last but one is the most like an empirical proposition. And it can understandably be called a synthetic *a priori* proposition.
>
> It can be said: unless you put the series of numbers and the series of letters side by side, you cannot know how many letters the word has.

Of course; no-one can be expected to understand the drift of such a sequence torn from its context: but it illustrates reasonably well the difficulties with which the reader has to contend. We have first two striking illustrations: but what exactly they are intended as illustrations of has to be gathered from the whole context in which they are embedded, bearing in mind that, if Wittgenstein had revised his text further, they might have been placed in a quite different immediate context, and bearing in mind also that what is printed here is only a selection, and the editors have not indicated where the omissions occur. There follows, in § 39, what can only be described as a series of musings. None of the observations it contains can possibly be gainsaid: our difficulty is to know what *force* they have. The four propositions are indeed obvious: what connection is this supposed to have with their bearing 'about essence'? What relation do such propositions have to others that are equally 'about essence' but are not at all obvious? In saying that they are 'about essence', is Wittgenstein merely borrowing a term from another philosophy in order to express the priori character which anyone would be likely to see as attaching to all four propositions, or does he have a sharp idea of essence? Do the propositions have *more* in common than what has already been stated, namely that they are obvious and are about essence? They are evidently all different, and differently used: but how great are we supposed to see these differences as being? The third proposition may understandably be called synthetic a priori: but can it justifiably be so called? To obtain any philosophical illumination from a passage like this, the reader must answer all these questions and more: and he must answer them not only to his own satisfaction, but in the manner that it is plausible that Wittgenstein intended them to be answered. As Frege said of his own posthumously published writings, not all of what is contained in this book is gold, but there is gold in it: but, in Wittgenstein's case, the mining operation is particularly arduous.

Michael Dummett

Michael Dummett is Fellow of All Souls College, Oxford, UK.

Geochemical handbook

Handbook of Geochemistry. Vol.2, Part 5: Elements La (57) to U (92). Edited by K.M. Wedepohl. Pp.1546. (Springer: Berlin, Heidelberg and New York, 1979). Looseleaf binder DM690; $379.50.

FOUR years have elapsed since the publication of Part 4 of the *Handbook of Geochemistry*, the longest gap in the production schedule, which has extended almost over a decade from 1969 to 1978. Part 4 is also by far the largest to appear, comprising more than 1500 pages. The original intention of the editorial board was that Volume 2 of the *Handbook* would ultimately contain approximately 2000 pages in four instalments, with publication completed several years ago. The much larger compilation and considerably extended publication time no doubt reflect many factors, not least the rapid growth of geochemical literature and the fact that all authors contributing to the project did not work at the same rate.

The *Handbook* was probably never intended, even in early days, for the individual purchaser. It is expensive to buy, now costing on the subscription price $785.60 (DM 1428.40), including Volume 1, which contained a series of chapters on geochemistry, geophysics and cosmochemistry. Volume 2 is, however, the principal expense. The dollar price for each part has, not surprisingly, risen over the years, but the price in German marks for each part has been held to the level at time of publication.

What does the purchaser receive for his money? In Part 5 he is presented with the pages to complete a large number of the chapters on individual elements, some of which were begun almost a decade ago. Several of these elements, notably phosphorus, sulphur, titanium, chorium, manganese, cobalt, strontium and molybdenum, are important geochemically, and virtually no information has been available for these elements until now in the *Handbook*. Although this lack of data has at times been frustrating, at least there is now the compensation that these chapters are the most up-to-date in the book. Many new pages carrying amendments that include additional information and the correction of scientific and printing errors, are also supplied with Part V.

Of the *Handbook* as a whole, once rearrangement of the entire loose-leaf system — not really a drawback, despite editorial concern — has been completed, the text represents the most comprehensive compilation of geochemical data so far attempted in one publication. The *Handbook* is logical in lay-out, with a high publishing standard of text (and the many tables and diagrams) maintained throughout. As more than 100 authors have been involved in the project, it is inevitable that the style, presentation and depth of coverage between, and even within, chapters will vary. The reader will have to accept this lack of uniformity, just as he will have to accept that much of the text is not up-to-date — the first manuscript was received in 1965. The editorial board now accepts that revision of earlier text to include more recent information is impossible, whatever its members might have felt individually or collectively in the euphoria at the start of the project. They must be relieved it is all over and it is perhaps unlikely that any one of them will embark again on such a large-scale project. The editors and authors should receive the congratultions and thanks of geochemists, first for successfully completing the *Handbook* and second for providing such a valuable authoritative base of data that will make the geochemist's professional life so much easier.

D.G. Murchison

D.G. Murchison is Professor of Organic Petrology and Past Reader in Geochemistry at the University of Newcastle-upon-Tyne, UK.

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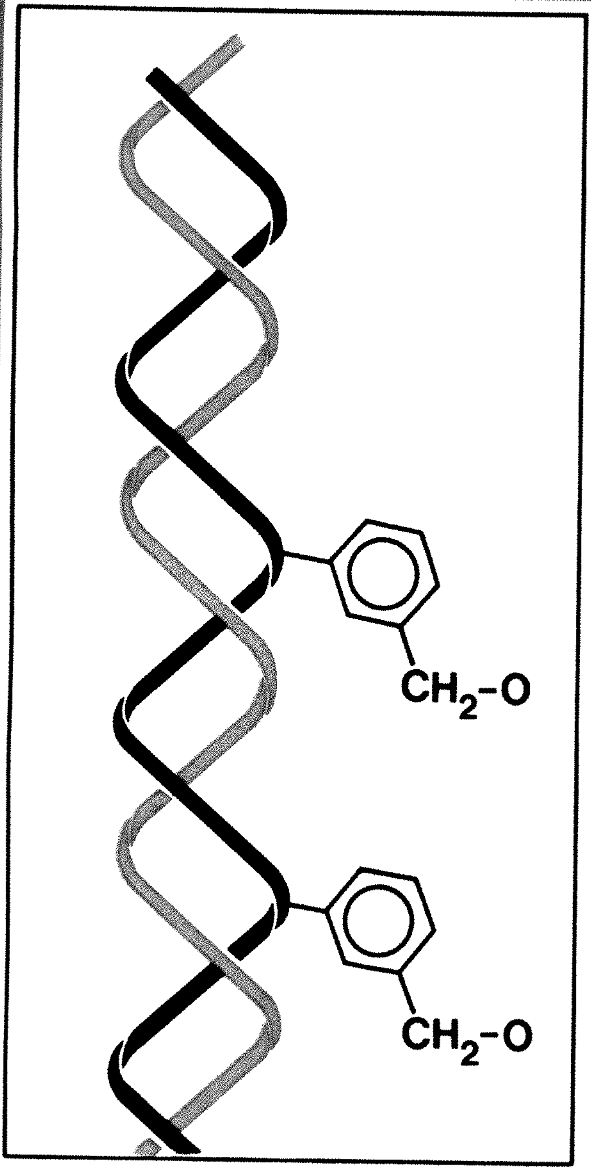
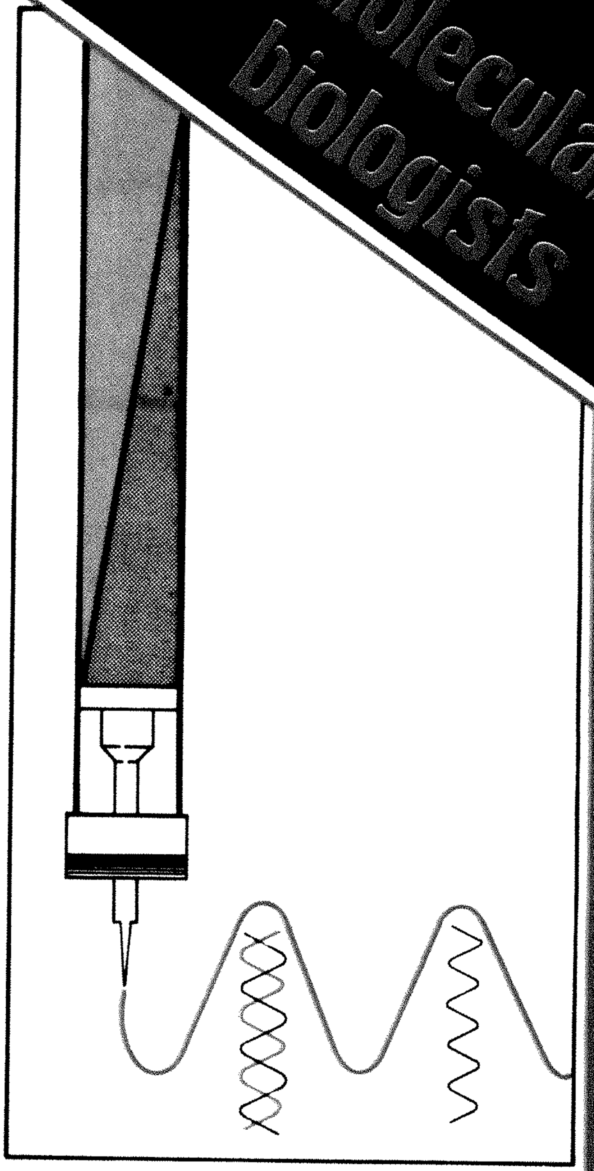
molecular biologists





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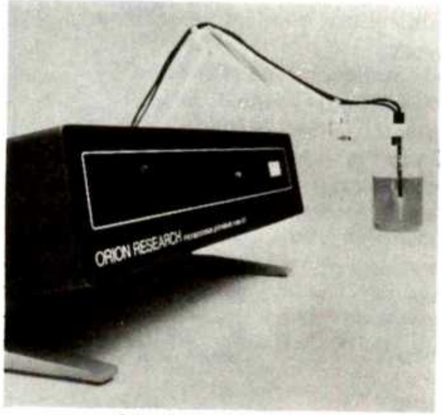
new products: electrodes in analysis

These notes, prepared from material provided by the manufacturers, are intended to give an outline of the range of products on the market. For more details use the Reader Enquiry Card bound inside the cover.

A feature on electrophoresis and immunoassay will appear in the 18 October issue.

ORION RESEARCH

The Orion range of ion selective electrodes offers electrodes for some 22 different chemical species, including fluoride, chloride, calcium, potassium, nitrate, ammonium and dissolved oxygen. Orion Research now offer eight new glass electrodes and one reference electrode. The eight sensing electrodes consist of four *p*H, two sodium, and two platinum electrodes for redox and Karl Fischer measurements. These high quality glass electrodes are designed to produce very fast, reliable response. All electrodes are rugged and deliver highly precise measurements time after time. The series provides versatility for a wide range of applications for every laboratory — *p*H, redox and sodium measurement. Included are electrodes for specialised applications. All electrodes are available with standard US connectors. Seven other connectors may be ordered for use with any major manufacturer's *p*H meter.



Orion Research model 811

The Orion model 901 is a digital specific ion meter that incorporates a microprocessor to provide direct readings of concentration. The 901 completely eliminates calibration curves, tables, or calculations in measurements with specific ion and gas-sensing electrodes. Results are calculated in real time and displayed with an accuracy that will satisy the most demanding research application — yet the 901 is simple enough to be used in all quality control applications. Eleven operating modes cover the full range of analytical methods used for performing electrode analyses. Analytical parameters are entered on the digital switches on the control panel, with standardisation and blank correction accomplished at the push of a button. Results are read directly from the LED display in the unit system of your choice.

With only a mode selector and a push-button for standardisation, the model 811 simplifies and automates every step in *p*H measurement except putting the electrode into solution. The microprocessor in the 811 contains a 'look-up' table of NBS buffer values as a function of temperature, ensuring correct *p*H standardisation. Error codes signal whenever an electrode is defective or has a low slope, or if the operator attempts standardisation with the wrong buffer. A stability indicator tells when the displayed reading should be recorded. The output of a platinum resistance thermometer is used to precisely correct sample *p*H readings for changes in temperature. The 1.5 cm high LED display gives readings precise to 0.001 *p*H units. Additional modes allow readings of electrode potential to 0.1mV, electrode slope to 0.1% of theoretical, and temperature to 0.1°C. In the standby mode the 811 becomes a digital clock. Other features of the meter include BCD output, Karl Fischer polarising current, and recorder output. Measurement with specific ion and gas sensing electrodes can be made using calibration curves, known increment tables, or titrations.

Circle No. 86 on Reader Enquiry Card.

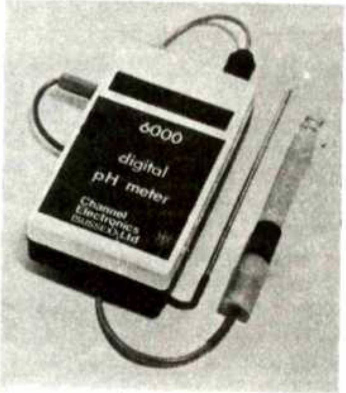
BORO LABS

Boro Labs of Aldermaston offer two distinct ranges of *p*H meters. For the user whose requirement is high accuracy, reliability and reproducibility Metrohm Ltd of Herisau produce instruments with both analog and digital readout systems, from a pocket size unit weighing only 450 g up to precision research instruments offering accuracy of 0.01 *p*H. Uniprobe *p*H meters offer economically priced, mains or battery powered accurate and reliable instruments, for both laboratory and field applications. They are particularly suitable for industrial use where the quality of their controls and the heavy duty case will withstand semi-skilled operation and considerable physical abuse. Boro Labs are also agents and stockists for the wide range of competitively priced *p*H electrodes manufactured by Russell *p*H of Auchtermuchty and can offer rapid delivery of the more popular types.

Circle No.87 on Reader Enquiry Card.

CHANNEL ELECTRONICS

The 6000 series of hand-held battery-driven *p*H/temperature meters from Channel Electronics (Sussex) Ltd offers fast and accurate measurement of both 0–14 *p*H, and temperature over the range -30°C to +150°C. Housed in a robust injection moulded case with 10.2 mm height LED readout the series is available in three basic models. The 6060 provides *p*H measurement to a resolution of 0.1 *p*H and the 6080 to a resolution of 0.01 *p*H. Temperature on both instruments is to a resolution of 1.0°C. A third instrument, the Model 6090 offers -999 to +999 mV with a resolution of 1.0 mV. Models 6060 and 6080 are supplied complete with combined plastic bodied *p*H electrode and steel bodied temperature compensation/measurement probe. Using standard MN1500 (manganese alkaline) batteries the 6000 series permits up to 35 h of continuous use or 60 h intermittent use. The instrument can be provided with type AA rechargeable cells plus a mains recharger unit. A further option is a carrying case with a separate pocket for the probes.

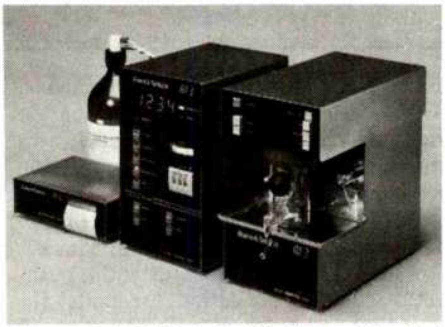


Other products from Channel Electronics include the model P101 portable *p*H meter for rapid determination of *p*H and millivolts. The 101 operates from two small universally obtainable batteries and features a chart recorder output socket. The MP 100 *p*H chart recorder is a miniature galvanometer chopper-bar recorder developed to provide a permanent record of *p*H level when used in conjunction with a *p*H meter having a d.c.output. The recorder has a calibrated chart width of 59 mm and is available with a choice of chart speeds of between $\frac{1}{12}$ and 480 inches per hr. The instrument is extremely rugged and has been designed for reliability, requiring no routine attention other than replacing the chart roll. The unit is available in a.c. or d.c. versions with a self-contained d.c. package for field use.

Circle No.88 on Reader Enquiry Card.

BAIRD & TATLOCK

The new AF3 direct reading Karl Fischer titrator from Baird & Tatlock utilises modern computer technology and a controlled impulse metering pump (instead of the usual burettes) as the two principal elements of a fully-automated, push-button system. (1) The AF3 comprises two basic modules:- The metering module which houses the pump and liquid handling system, and automatically takes the correct volume of reagent straight from a standard 1 or 2 litre Winchester bottle. No reservoir bottles, volumetric glassware or syringe burettes are involved. And (2), the titration module, which contains the electronics and main controls, automatically displays the results directly as a digital readout in mg of water to the nearest 0.11 mg. No time consuming calculations are involved. An ancillary printer unit is available as an optional extra. Apart from the major advantage of offering a direct water content display without calculation, the auto metering system also provides many other operational benefits. A 'standby' facility enables the titrator to be kept in a 'neutral' condition immediately ready to titrate any sample offered to it. Sensors set up fault alarms and take appropriate action if the titration vessel becomes overfilled or reagent is spilled on the operating platform. A special timer computes metering pump operation and gives warning of routine replacement. 'Prime' and 'Clear' buttons enable the unit to be brought into operation rapidly, and just as easily closed down at the end of a working period. A low voltage magnetic stirring facility gives constant speeds under all conditions. All results are fully compatible with earlier Karl Fischer titrometic units.


The new Baird & Tatlock AF3 direct reading Karl Fischer titrator

Circle No. 89 on Reader Enquiry Card.

KANE-MAY LTD

Advances in microelectronics have made possible an extremely reliable pocket-sized, digital pH meter, the Accuchem, manufactured by Kane-May Ltd. Accuchem allows for the instantaneous measurement of pH values over the full scale of 0 to 14 pH with a 0.01 resolution. This portable pH meter provides large 13-mm readings on an LCD display. Operating from a single 9 V PP3 battery, the Accuchem will allow field use for well over 100 h continuous operation. The instrument is available with a rugged combination electrode which is designed to withstand hard industrial and site use. A small unbreakable carrying case contains both the instrument and electrode.

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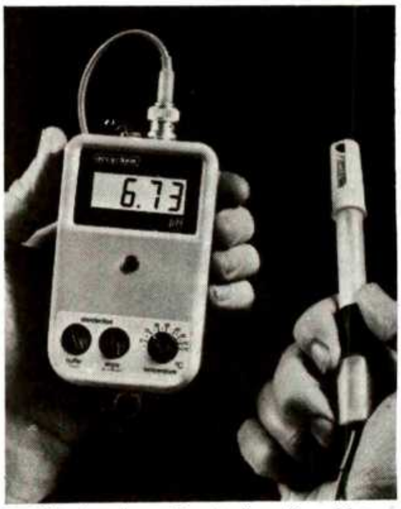

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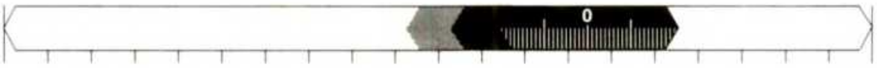

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Scientific findings challenge EPA ban

DEBATING SCIENTISTS OF 8 NATIONS AGREE PESTICIDE 2,4,5-T IS SAFE!

- *poses no cancer hazard to humans or animals*
- *does not cause abortions in women (as claimed)*
- *poses no practical environmental harm*

Safe...that's the consensus of 59 scientists. Top world experts on pesticides who journeyed to Washington, D.C. recently from seven countries and 20 U.S. States to 'dispute and resolve' the controversy surrounding the currently banned 2,4,5-T, the pesticide widely used to protect forests, cattle and weeds.

Scientific findings of the unique, first-of-its kind Dispute Resolution Conference sponsored by the American Farm Bureau Federation run counter to the position of the Federal EPA which placed a temporary ban on most uses of the pesticide in March.

To those who still believe 2,4,5-T should be banned we commend the just released Conference report. Especially the scientific conclusion on the Alsea, Oregon epidemiology study on which the EPA based its action to ban. The scientists agreed that the report has such deficiencies that no conclusions are possible with regard to alleged abortion-causing effects of 2,4,5-T.

We compliment the scientists who took the necessary time to resolve issues now restricting the use of this important agricultural tool. The participants came from the fields of medicine, toxicology, chemistry, ecology, agriculture. They represented the scientific concern of Canada, Italy, Sweden, New Zealand, Switzerland, France, Germany and the U.S.A.

But we wonder why many of the ever-vocal minority who seek a final end to the use of 2,4,5-T and other pesticides *declined to attend* or to join in the scientific dispute ... certainly an ideal forum for arguing their case with the world's scientific experts.

The full Dispute Resolution Conference report has been mailed to Members of Congress and other government officials ...we urgently direct their attention to this decisive, truly scientific consensus ...that 2,4,5-T is safe when properly applied.

It's important to understand that there is still no *scientific* reason to prevent the pesticide's use by literally hundreds of thousands of Americans who have depended on it for 30 years...in growing their crops, cattle, timber. For more information on the Conference findings, write American Farm Bureau Federation, 225 Touhy Ave., Park Ridge, IL 60068.

DOW*

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Circle No. 26 on Reader Enquiry Card.

SEMAT

Semat (UK) now offer both digital and analog *p*H meters available with a wide range of compatible electrodes. The digital meters are available in LED or LCD forms and are designed for both laboratory and field work.

The CD60 with its 13-mm liquid crystal display provides an accuracy of 0.02 *p*H units when used with a standard electrode. Battery powered, and provided with a tough, durable carrying case, the CD60 is a compact, simple to operate instrument ideally suited to outside measurements, yet its versatility allows equal application to laboratory work. Attachment of the PS100 adapter converts it to mains operation. For *p*H and/or mV work, Semat produce the CD300. Push button controls on the front panel select the readout range to be either 0 to 14 *p*H, 0 to 999mV or 0 to 9.99V. Temperature compensation and buffer standardisation are provided with simple slider controls which are also front panel mounted.

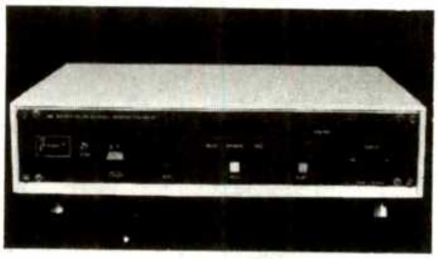
SEMAT CD300 combination digital pH/mV meter

Semat (UK) also stock a wide range of electrodes including glass, reference, combination, general purpose and gel filled, all compatible with any of their meters. They are happy to advise on any *p*H related problem and assist in optimum electrode choice.

Circle No. 91 on Reader Enquiry Card.

EDT RESEARCH

The ATC 700 automatic titration system from EDT Research comprises a motor-driven burette, analytical cell with sensor and automatic sample changer all connected to a microprocessor-based control console. The instrument is capable of performing repetitive titrations, using potentiometric sensing, completely unattended with high precision and accuracy. It is extremely simple to operate, having very few external controls, and the microprocessor takes care of sample selection, titrant addition, endpoint detection and results display and print-out. The titrator has been designed specifically for use with ion-selective electrodes to make allowance for the somewhat slower responses of these sensors. Various error detect functions within the instrument will inhibit analyses if the indicator or reference electrodes are malfunctioning, thus eliminating the possibility of incorrect results. The ATC 700 can be used for a wide variety of analyses including: acid-base titrations using strong or weak acids and bases; precipitation titrations such as chloride and sulphate determinations; complexometric titrations such as calcium, magnesium and zinc determinations; and redox titrations such as iron and permanganate measurements.



EDT Research ATC 700 control console

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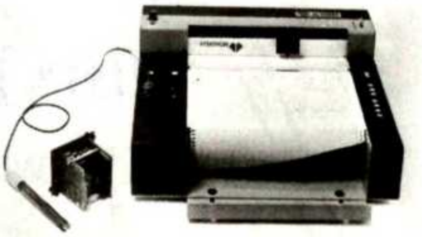
VITATRON

A novel product in the *p*H field is the Vitatron model 2001 chart recorder, which can be supplied with the high impedance input module which will accept direct input from a *p*H electrode, without the need for a *p*H meter. The signal which drives the 2001 enters via an input module, which is plugged into the recorder chassis. A variety of input modules is available, including the new *p*H input module. This will take a combination *p*H electrode fitted with a BNC plug. The recorder reads *p*H from zero to 14 across the chart, and there is an expanded *p*H range of 3 *p*H units for more precise measurements. To obtain the 2001 recorder brochure detailing this technique, circle the number below.

Circle No.93 on Reader Enquiry Card.



Vitatron 2001 chart recorder with pH electrode

TECATOR

A new analyser — Striptec — for precise trace metal determinations is being introduced by Tecator. Striptec uses new technology that gives a very large dynamic measuring range, with no special preparatory steps. The instrument is capable of simultaneous measurements of several metals with high precision at very low concentrations (parts per 10^9. Typical applications are tap water, waste water, seawater, beverages, wine and urine. In combination with Tecator's programmable digestion systems for multi-sample preparation Striptec allows convenient and reproducible conditions for the measurement of metals not only in liquids and inorganic materials, but also in all kinds of food-materials.

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The Striptec system: electrode stand, processing unit and recorder

RUSSELL

The Scottish-based *p*H electrode manufacturer, Russel *p*H, are improving supply and service to customers in the South of England through an arrangement with Semat (UK) who will stock and market the Russell electrodes range. Semat are now able to supply from stock, both instruments and electrodes as a complete package. A further advantage will be an ability to supply replacement electrodes for all makes of *p*H meters whether of British, European or American manufacture due to the wide and versatile range produced by Russell *p*H. This range includes glass single and combination electrodes, low-resistance electrodes having very fast response, and reference, metal and polymer-bodied types.

As well as laboratory electrodes, units for industrial plant applications are also available including disposable and replacement combination types, and last but not least, the Select range of lead-less electrodes recently released. These lead-less electrodes are fitted with a precision-made moisture proof cap/socket arrangement and utilise a mating plug/cable assembly supplied with correct meter termination(s). This lead once fitted to the *p*H meter becomes a permanent fixture and greatly simplifies subsequent electrode replacement.

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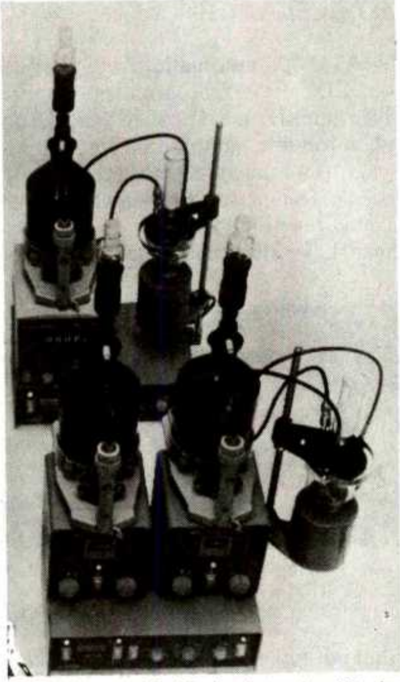

Two of the range of Karl Fischer titrators from Metrohm.

BRINKMANN

Brinkmann Instruments Inc. a subsidiary of Sybron Corporation now have available a new brochure describing five Metrohm Karl Fischer titrators which perform moisture determinations efficiently. These titrators can provide every laboratory with the versatility it needs at a low price, whilst maintaining quality, accuracy and precision. All models measure moisture concentrations as low as 10 p.p.m. and as high as 100%, perform back titrations, feature 'snap-in' burette units for rapid change of solvent and reagent, and use specially designed titration vessels that allow sample introduction without exposure to atomspheric moisture.

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METTLER

Electrodes are used as indicators in the Mettler range of automatic titrators. The latest product in the range is the model DL40 MemoTitrator, a microprocessor controlled instrument with 10 different titration modes and extensive computation facilities. The 10 modes include titrations to a pre-selected endpoint (absolute or relative), equilibrium titrations, recording titrations, incremental titrations, Karl Fischer determinations, precision dispensing, *p*H and *p*X measurements, back titrations with automatic calculation of results, manual titrations and automatic electrode calibrations. Endpoints can be indicated by potentiometric, voltammetric or photometric sensors. By connecting an electronic analytical or top-loading balance, and a printer or recorder, a complete titration station can be set up in a minimum of space. One novel function is

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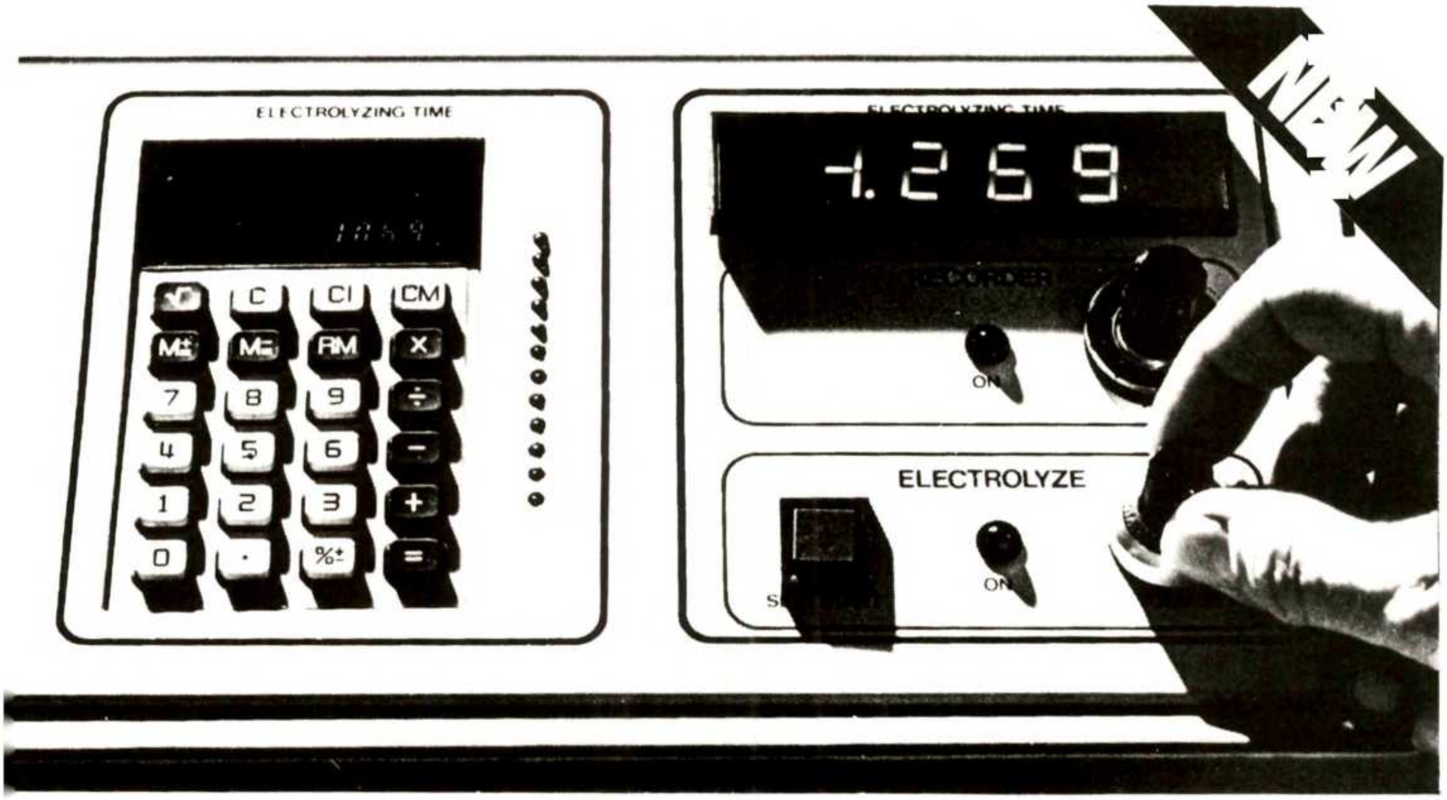
The new approach to heavy metal analysis...



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Step forward with the latest development from Tecator!

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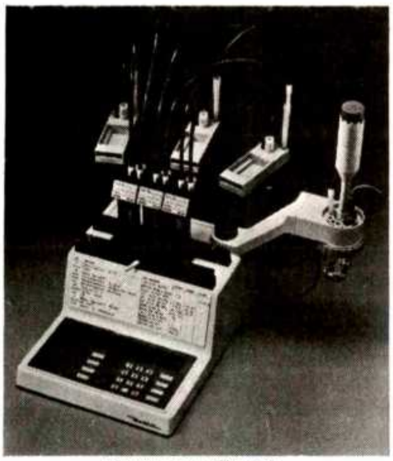

DL40 Memo Titrator

the instrument's 'learn' mode. If the operator is not sure of the best method of titration, the MemoTitrator can be asked to suggest a method. This is done by subjecting the sample to a special incremental titration in which its approximate content, the best possible titration principle and the optimum parameters are determined.

Mettler also offer the model SR10, a fully automatic instrument into which operators can load up to 96 samples for analysis by a choice of four types of titration. Once set up, the instrument works automatically and without attention. Though endpoints in automatic titration are normally sensed by electrodes, there is often a need to use a colour change as indication, and Mettler have recently introduced the model DK18/181 Phototitrator. This is a scanning instrument which can be used throughout the visible spectrum to respond to endpoint colour changes, using the results to control any automatic titrator from the Mettler range.

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MSE

As agents in the UK for Orion Research and Mettler, MSE Scientific Instruments can offer a wide choice of analytical methods using electrodes. New to the UK is the Mettler DL40 MemoTitrator which features a facility for storing the parameters necessary for 10 standard titration modes. The Orion range includes the SS20 ionised calcium analyser and type SS30 sodium and potassium analyser for blood determinations, and the 417 skin chloride analyser. A recent introduction from Orion is the GX series of epoxy-body 'unbreakable' gel-filled electrodes.

MSE can supply the module required to convert Vitatron 2001 chart recorders to use as a continuous monitor of *p*H by direct link to a *p*H electrode. This module is also available fitted with a suitable electrode, such as the Orion model 9005.

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PROBION

Probion Ltd are able to produce specially constructed *p*H glass probes made of high quality membrane glasses at reasonable cost. New advances in the design of autoclavable *p*H combined glass-reference electrodes have now made it possible to sterilise the *p*H sensors *in situ* at high temperatures. Probion also market a wide range of *p*H electrodes which can be used with any *p*H equipment of whatever make. Some are capable of measuring samples as small as 20 ml, others — sheathed in inert plastic — have an immersion length of 2 m. Recent developments include miniature probes for use in isoelectric focusing gels, and glass probes for the measurement of P_{CO_2} and dissolved oxygen.

Probion collaborate closely with their customers in developing *p*H electrode systems for specialised uses.

Circle No. 99 on Reader Enquiry Card.

BECKMAN

Beckman have introduced three new SelectIon analysers for fast, accurate ion-selective measurements. The product range now includes the three new analysers, more than 20 electrodes, and related supplies and reagents. Each of the instruments measures specific ions down to fractional milligrams per litre concentrations. Electrodes are availble to measure ammonia, bromide, cadmium, calcium, carbon dioxide, chloride, copper, cyanide, fluoride fluoroborate, hydrogen, iodide, lead, nitrate, nitrogen oxide, perchlorate, potassium, silver, sodium, sulphide, thiocyanate and water hardness. Beckman plan to add more electrodes at later dates.

The new SelectIon analysers from Beckman

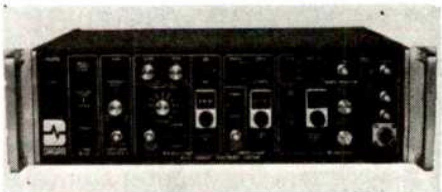
SelectIon systems are especially suited for applications in water purification, sewage treatment, plating, agriculture, food processing and analysis, and biomedical research. The SelectIon range includes the SelectIon 5000 ion analyser, a microprocessor-based instrument; the SelectIon 2000, an analog instrument; and the Monitor II system, a rugged portable analyser. The research-grade SelectIon 5000 ion analyser features computer programming that displays words and symbols to communicate with the user to make operation simple. It signals when steps are forgotten or errors are made. Specific ion computations are done automatically, and final answers are digitally displayed. Steps such as standardisation and calibration are simple push-button operations. A printer out-put enables permanent recordkeeping and sequential step recording. The SelectIon 2000 combines advanced electronic test circuits with ion-selective operations for several routines for direct concentration measurement as well as *p*H, expanded-scale *p*H and mV. Multiple controls on the analyser provide fine tuning of determinations for increased accuracy. The SelectIon 2000 uses all ion-selective electrodes through sensitivity and polarity changes. Because of wide-range standardisations, silver or sulphide electrodes with large potential offsets can be used. Two-point calibrations can be made with standardisation at any point on the scale. The slope control allows calibration for direct concentration readout and provides sensitivity change for different electrode responses. A midscale value control lets the user shift the calibrated range without re-calibrating. The SelectIon 2000's expanded *p*H and mV readout mode increases accuracy. Its absolute mV mode performs titrations easily. The mirror-backed analog display helps the user to anticipate endpoints. Another significant feature is the electronic test circuit that verifies proper instrument function.

The Monitor II system is a fully portable unit designed for factory, laboratory or field applications.

Circle No.100 on Reader Enquiry Card.

DAGAN

The first commercially available single electrode voltage clamp, current clamp and pre-amplifier is now available from Dagan Corporation of Minneapolis. Pictured is the model 8100 single electrode system. Voltage clamping is one of the most powerful methods available for studying the electrical events occuring in cell membranes. Voltage clamping with a single electrode is accomplished by alternating between record and voltage or current maintenance modes. Use of a single electrode eliminates the difficulties of a second electrode such as maintaining a healthy cell or electrical coupling, and also, the minute cell size might dictate the use of only one electrode.



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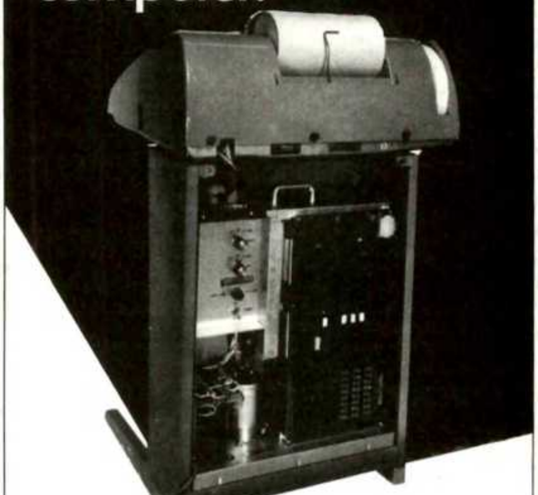

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ROTH SCIENTIFIC

Metrohm automatic titration equipment is supplied by Roth Scientific of Farnborough, Hants. Systems available include the type E576 automatic potentiograph in series with an E365B Conductoscope and E575 Dosimat, and the E535 general purpose Multidosimat coupled to an E549 titration stand and the E518 conductivity meter in series with E576 Potentiograph. Metrohm automated titration equipment provides proven reliability, accuracy and speed of handling for titrimetric reactions.

Circle No.102 on Reader Enquiry Card.

RADIOMETER

In August Radiometer of Copenhagen announced their new "80 Series", range of titration equipment which is a completely integrated modular system offering many different approaches to your titration problems. The Series 80 begins with simple endpoint systems through to curve recording (including first differential) on to sophisticated microprocessor controlled endpoint searching systems (plus curve print-out as an option). Other features are automatic sample preparation and presentation for single or multiple end-point determinations (including back titrations), so complete automation is possible. Series 80 systems are interchangeable with the 60 Series systems currently in use. The major advance in the Radiometer system has been the introduction of the ABU 80 microprocessor controlled Autoburette. Simply slide in the correct burette in its easy-load cartridge and the ABU 80 takes over automatically setting the stop-cock to the correct position and following a pre-programmed routine to fill and purge the burette ready for use. At the push of a button any of the burette modules (2.5 ml, 10 ml and 25 ml) can be made to operate at 1/10 of their normal operating volume, giving the operator a very wide choice of working volumes. All glassware and liquid flow systems are easily accessible but kept under cover protecting them from accidental damage or the effects of light. A large clear LED volume display with automatic decimal point setting gives clearly visible, unambiguous readings. The ABU 80 will 'stack in' with the other modules leaving the maximum possible bench space to work on.

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INSTRUMENTATION LABORATORY

The new I.L.617 Blood Gas Laboratory provides the blood gas data vital to advanced patient care. The two major components of blood gas analysis — acid/base status and oxygen status — are both analysed comprehensively. For the rapid assessment of acid/base balance, the I.L.617 determines and prints out *p*H, P_{CO_2}, base excess, total CO_2 and bicarbonate. To clearly define oxygen status, it provides P_{O_2}, total haemoglobin, % oxyhaemoglobin, % carboxyhaemoglobin, % methaemoglobin and O_2 content. Operation is simple — you present blood to the system and approximately 90 s later all data is printed out in as many copies as you wish. Cleaning and recalibration cycles follow automatically.

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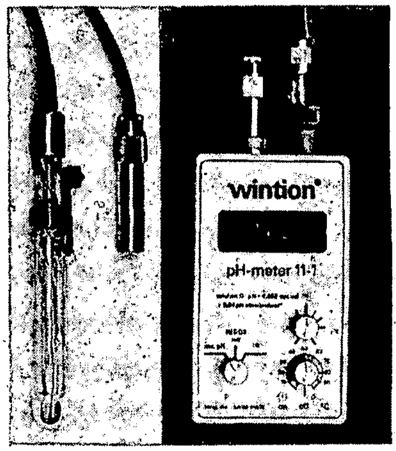
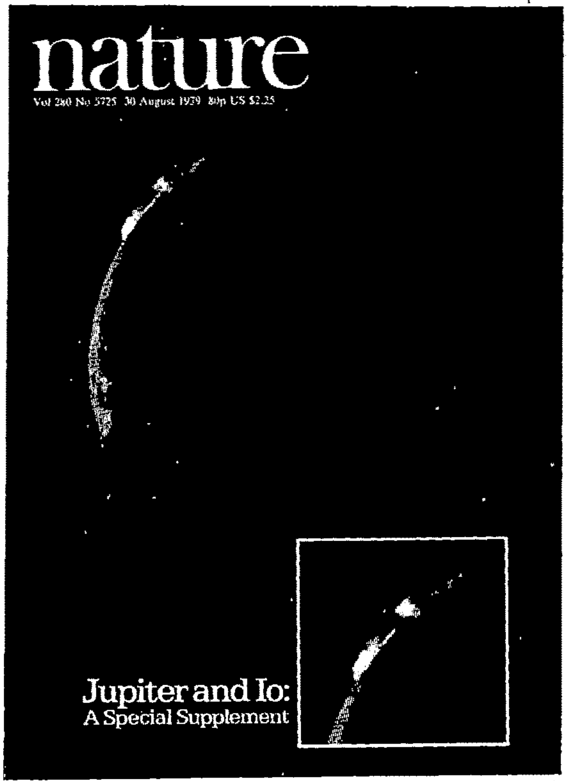


Wintion introduce a new range of direct reading pH meters which also measure mV and pX for a wide range of ions. Circle No.85 on reader enquiry card for more details on Wintion products



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announcements

Meetings

27-28 September, **Annual Meeting of the Society of Low Temperature Biology,** London (Dr B. Fuller, Academic Department of Surgery, Royal Free Hospital, London NW3, UK).

8-10 October, **6th Biennial Symposium on Turbulence,** Rolla (Norma Fleming, Coordinator Continuing Education University of Missouri-Rolla, Rolla, Missouri 65401).

17-19 October, **The Use of Microprocessor-Based, 'Intelligent' Machines in Patient Care,** Maryland (Dr Henry S. Eden, Department of Health, Education and Welfare, Public Health Service, National Institutes of Health, Bethesda, Maryland 20014).

18 October, **Fracture Mechanics,** London (The Meetings Secretary, The Institution of Metallurgists, Northway House, Whetstone, London N20, UK).

30 October, **Onshore Exploration and Production in the United Kingdom,** London (The Secretary, The Institution of Mining and Metallurgy, 44 Portland Place, London W1, UK).

4-11 November, **10th International Congress of Allergology,** Jerusalem (Dr Israel Glazer, Chairman, Organising Committee, 10th International Conference of Allergology, Tel Aviv, Israel).

6-7 November, **The Impact of New Technologies on Publishing,** Luxembourg (J.M. Gibb, Commission of the European Communities, DG X111, P.O. Box 1907, Luxembourg).

5 December, **Electric Arc Steelmaking for the 1980's,** Sheffield (The Meetings Secretary, The Institution of Metallurgists, Northway House, Whetstone, London N20, UK).

11-12 December, **Environmental Effects of Utilising More Coal,** London (Council for Environmental Science and Engineering, Conference Office, c/o The Institute of Biology, 41 Queen's Gate, London SW7, UK).

1980

23 January, **The Integrity of Sprayed Coatings,** Birmingham (The Meetings Secretary, The Institution of Metallurgists, Northway House, Whetstone, London N20, UK).

10-14 February **EMBO Workshop of Drug Receptors in the Central Nervous System,** Rehovot (Mrs Chava Bohak, Secretary, EMBO Workshop, Department of Neurobiology, The Weizman Institute of Science, Rehovot, Israel).

27-28 March, **13th International TNO Conference on Biotechnology,** Rotterdam (TNO Information Department, 148 Juliana van Stolberglaan, 2595 The Hague, Holland).

31 March-3 April, **Envirpnmental Degradation of High Temperature Materials,** Isle of Man (The Meetings Secretary, The Institution of Metallurgists, Northway House, Whetstone, London N20, UK).

11-15 April, **Internation Conference on Endosymbiosis and Cell Research,** Tübingen (Dr W. Schwemmler, Freie Universität Berlin, Institut für Pflanzenphysiologie und Zellbiologie, Königin-Luise-Str. 12-16a, BRD 1000 Berlin 33, DDR).

13-17 April, **Neurological Mutants Affecting Myelination. Research Tool in Neurobiology,** Seillac (Dr N.A. Baumann, Laboratoire de Neurochimi INSERM U.134, Hôpital de la Salpêtrière, 47 bld de l'Hôpital, 75634 Paris Cédex 13, France).

23-27 June, **Conference on Precision Electromagnetic Measurements,** Braunschweig (Professor H. Capptuller, Physikalisch-Technische Bundensanstalt, Bundesallee 100, D-3300 Braunschweig, FRG).

8-11 September, **6th International Conference on Gas Discharges and their Applications,** Edinburgh (The Press Office, Institute of Electrical Engineers, Savoy Place, London WC2, UK).

7-12 September, **IUPAC International Symposium on Macromolecules,** Florence (Fondazione Giovanni Lorenzini, Via Monte Napoleone 23, 2021 Milan, Italy).

8-10 September **Photoelectrochemistry discussion,** Oxford (Dr M.D. Archer, Department of Physical Chemistry, University of Cambridge, Lensfield Road, Cambridge, UK).

8-11 September, **Dielectrics conference,** Canterbury (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1, UK).

8-12 September, **5th International Symposium on Inhaled Particles,** Cardiff (D.A. Phillips, Joint Organising Secretary, MRC Pneumoconiosis Unit, Llandough Hospital, Penarth, Glamorgan, UK).

8-12 September, **7th World Conference on Earthquake Engineering,** Instanbul (Aybars Gürpinar, Secretary 7 WCEE, Yüksel Caddesi 7/B, Ankara, Turkey).

9-11 September, **3rd IMA Conference on Control Theory,** Sheffield (Institute of Mathematics and its Applications, Maitland House, Warrior Square, Southend-on-Sea, Essex, UK).

10-12 September, **Cellular Responses to Mutagens and Carcinogens,** Brighton (Prof. R.J. Cole, (B.S.C.B./U.K.E.M.S. Symposium), University of Sussex, Falmer, Brighton, UK).

15-19 September, **10th European Solid State Device Research Conference,** York (The Institute of Physics, 47 Belgrave Square, London SW7, UK).

15-19 September, **3rd International Symposium on Loss Prevention and Safety Promotion in the Process Industries,** Basle (P.O. Box 141, CH-4007 Basle, Switzerland).

17-19 September, **Eurotunnel '80, Conference,** Basle (The Secretary, Institution of Mining and Metallurgy, 44 Portland Place, London W1, UK).

18 September-2 October, **International Symposium on Forest Seed Storage,** Ontario (Ben Wang, National Tree Seed Centre, Petawawa National Forestry Institute, Ontario, Canada).

22-28 September, **3rd International Colloquium on Physical and Chemical Information Transfer in Regulation of Reproduction and Ageing,** Varna (Dr J.G. Vassileva-Popova, Department of Biophysics, Bulgarian Academy of Sciences, 1113 Sophia, Bulgaria).

22-27 September, **International Practical Post-Graduate Course on Pain Therapy,** Vicenza (Dr Ruggiero Rizzi, Ospedale Regionale, 36100 Vicenza, Italy).

23-25 September, **1st International Conference On-Line Surveillance and Monitoring of Plant Integrity,** London (Miss J Bovier, Conference Secretariat, Society of Chemical Industry, 14 Belgrave Square, London SW1, UK).

24-28 September, **EMBO-EMBL Workshop, on X-Ray and Neutron Scattering of Biological Structures,** Hamburg (Professor H. Stuhrmann, EMBL c/o DESY, Notkestrasse 85, D-2000 Hamburg 52, FRG).

26-28 September, **Inorganic and Organic Pollutants in the Troposphere and Natural Waters,** Belfast (W.J. Swindall, Department of Chemistry, The Queen's University, Belfast, UK).

29 September-3 October, **Assembly on Current and Future Oceanography,** Massachusets (Dr P.G. Brewer, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543).

6-10 October, **5th International Conference on Small Angle Scattering,** Berlin (Professor R. Hosemann, Fritz-Haber-Institut der MPG, Teilinstitut fur Strukturforschung, Faradayweg 4-6, 1000 Berlin 33, E. Germany).

1981

17-19 March, **International Conference on Telecommunication Transmission,** London (Institution of Electrical Engineers, Savoy Place, London WC2, UK). WC2, UK).

5-11 July, **6th International Conference on Protozoology,** Warsaw (Dr S.L. Kazubski, Department of Cell Biology, M. Nencki Institute of Experimental Biology, 3 Pasteura Street, Warsaw 22, Poland).

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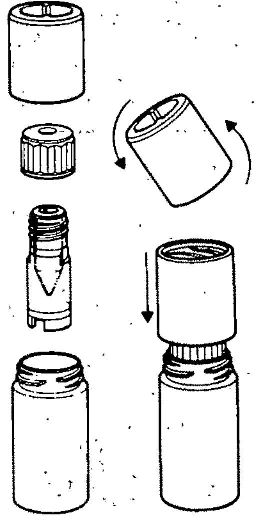

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ICE AGES

M

: Solving the Mystery
Professor John Imbrie and Katherine Palmer Imbrie

May 1979 £6.95 224pp 0 333 26767 2 illustrated

Knowledge that the world once endured an ice age has been widespread for more than a century. Indeed, the concept is now so familiar that nearly every winter storm prompts dramatic headlines: Is a new ice age upon us?

This book tackles that burning question and attempts to unfold, for the interested general reader and those with a more academic interest, the entire saga of the Ice Age 'mystery' – why ice ages occurred, what they were like, and when the next one is due.

It is written by one of the principal scientists on the multi-million dollar CLIMAP project which set out to study changes in the climate of the earth over the past 700,000 years. Out of the group's analytical work came an exciting by-product: confirmation of the correctness of one of the several theories of what causes ice ages to occur. The theory that was confirmed, and is now explained along with many others in ICE AGES, is the Milankovitch, or astronomical theory which sees periodical glaciation as the result of three regular movements the earth makes as it travels around the sun: changing ellipticity of the earth's orbit, "wobble" of the earth's axis, and the changing tilt of the earth's axis.

"ICE AGES is fascinating in its scientific interest and also as a detective story. I recommend it to all." Dr. William F. Libby, Nobel Laureate.

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For further information or copies of the above please contact: The Macmillan Press Ltd.,
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APPOINTMENTS VACANT

UNIVERSITY OF BRISTOL

DEPARTMENT OF BIOCHEMISTRY

Applications are invited for a

POSTDOCTORAL ASSISTANT (Range 1A)

funded by an M.R.C. Project grant to study the biosynthesis of the erythrocyte membrane anion transport protein. The project will investigate the mechanism of incorporation of this integral protein into membranes, both in intact cells and in an mRNA-dependent cell free protein synthetic system. Applicants with previous experience in the area of nucleic acids and protein biosynthesis preferred. The post is for 3 years at a salary of £4,910 to £5,199 to £5,480 per annum. (Scale effective from 1st October, 1979 and subject to further review). The post will be available from October/November 1979.

Applicants should send their curriculum vitae and the names of two academic referees as soon as possible to Dr M.J.A. Tanner, Department of Biochemistry, University of Bristol, Bristol BS8 1TD. 3239(A)

SIMON FRASER UNIVERISITY

Burnaby, British Columbia, Canada.

DEPARTMENT OF BIOLOGICAL SCIENCES

DIRECTOR OF MASTER OF PEST MANAGEMENT PROGRAM

Applications are invited for the vacant position of Director of the Master of Pest Management (MPM) program and related activities at Simon Fraser University.

The appointment will be at the rank of Associate Professor or Professor, with level and salary commensurate with training and experience. The successful candidate should have an established professional and academic reputation in pest management and a current research program in at least one aspect of pestology. Responsibilities will include the development and organisation of the MPM program as well as research and teaching in the individual's area of expertise. The position is available as of 1 September, 1979, subject to budgetary approval. The current salary range for the Associate Professor and Professor rank is, respectively, $25,636 to $41,018 and $31,552 to $55,216 per annum.

Applications, including an up-to-date curriculum vitae, copies of representative recent publications and the names of at least three referees, should be sent to: Dr M Mackauer, Chairman, Department of Biological Sciences, Simon Fraser University, Burnaby, BC, Canada, V5A 1S6 from whom further information may be obtained. Applications will be received until 31 October, 1979 or until the position is filled. W341(A)

POPULATION GENETICIST

The Department of Biology at the University of California, Riverside invites applications for a faculty position in population genetics beginning September 1, 1980. Candidates must have evidence of ability to initiate and sustain a productive, high-quality research program and show enthusiasm and skill in teaching at both the undergraduate and graduate levels.

Teaching duties may include introductory biology, genetics, and upper division and graduate courses in population genetics. Appointment will be made at the Associate Professor level. Applicants should send curriculum vitae, statement of teaching and research interests, reprints and letters from three referees.

The University of California is an equal opportunity and affirmative action employer. Applications are invited from all qualified individuals, especially women and members of minority groups.

Send applications to: Professor R. Ruibal, Chairman, Department of Biology, University of California, Riverside, California 92521. W 370(A)

UNIVERSITY OF NEWCASTLE UPON TYNE AND NEWCASTLE AREA HEALTH AUTHORITY (Teaching)

CHAIR OF EXPERIMENTAL NEUROLOGY in the DEPARTMENT OF NEUROLOGY

Applications are invited for the Chair of Experimental Neurology, which is endowed by the National Fund for Research into Crippling Diseases. Medically qualified applicants should hold a higher medical degree and have extensive research experience in adult or paediatric neurology or in neuropathology or in related disciplines, preferably of particular relevance to neuromuscular disease. The appointment will take effect from 1st March 1980 or as soon as possible thereafter.

The successful candidate, if a registered medical practitioner, will be accorded Honorary Consultant status with the Newcastle A.H.A. (T). Salary in accordance with the Professorial (Clinical Consultant) or Professorial (Non-Clinical) scales, with membership of the appropriate University superannuation scheme.

Further particulars may be obtained from the Senior Assistant Registrar, The Medical School, University of Newcastle upon Tyne, Queen Victoria Road, Newcastle upon Tyne, NE1 7RU, with whom applications (15 copies) giving the names of not more than three referees, must be lodged not later than 31st October, 1979.

(Applicants from outside the British Isles may submit one copy only). 3218(A)

THE POLYTECHNIC OF NORTH LONDON

DEPARTMENTS OF SCIENCE AND TECHNOLOGY

RESEARCH ASSISTANT VACANCIES

There are possible vacancies for Research Assistants in the following areas: Biology, Chemistry, Computing, Geology, Polymer Science and Technology, Physics, Statistics.

Candidates should have a good honours degree in a relevant subject. Research Assistants are required to assist with the teaching of the Polytechnic for up to six hours per week and normally register for a higher degree of the CNAA.

Salary Scale: £3,087 to £3,261 inclusive of London Allowance (under review).

Application forms and further details may be obtained from the appropriate Head of Department. 3244(A)

OXFORD UNIVERSITY

DEPARTMENT OF BOTANY & LABORATORY OF MOLECULAR BIOPHYSICS

Applications are invited for the post of Research Assistant grade 1A supported by a grant from the Agricultural Research Council for research into the structure of ribulose bis-phosphate carboxylase (RuBPC). The grant is for three years starting on 1 November 1979 and the salary will be £5,048 to £5,321 to £5,591. The applicant should have obtained his PhD or DPhil and should already be familiar with enzymological and physical chemical methods. The successful applicant's work will be to isolate and crystallise RuBPC for X-ray structure analysis.

Applicants should apply in writing by 12 October to the Administrator, Botany Department, Oxford University, South Parks Road, Oxford, and include a curriculum vitae and the names of two referees. 3250(A)

Public Health Laboratory Service

Deputy Curator

(Principal Grade Microbiologist)

National Collection of Type Cultures Central Public Health Laboratory, Colindale

Colindale Avenue, London NW9 5HT

Applications are invited from experienced science graduates in Microbiology, preferably with a higher degree, for the whole-time post of Deputy Curator. The primary function of the NCTC is to hold and supply authenticated bacterial cultures of medical or veterinary importance for education, control, assay, taxonomic and other purposes to PHLS laboratories, Universities, Medical Schools, Hospitals and Industry. Other functions include the provision of an identification service for bacteria of medical relevance that are difficult to classify, research on bacterial taxonomy and the provision of freeze-drying facilities for other PHLS laboratories.

The total staff establishment is 23. The Deputy Curator will assist the Curator in directing and developing all aspects of NCTC work.

Further details of the post are available from the Curator (Dr. L.R. Hill) and the laboratory may be visited by arrangement (Tel. 01-205 7041. extension 236).

Salary: Principal Grade Microbiologist £7,995 to £10,458 + £354 London Weighting, increasing to £9,129 to £12,306 + £354 London Weighting on 1 January, 1980. Other terms & conditions of service generally as for appointment in the NHS.

Applicants should submit a curriculum vitae, naming three referees, to the Secretary, PHLS Board, 61, Colindale Avenue, London NW9 5EQ. Closing date: 12th October, 1979.

PHLS

3247(A)

Researchers

Overseas Assignment

The Kuwait Institute for Scientific Research is a rapidly expanding institute for applied science and technology. We are currently seeking Researchers in the following disciplines to work in our Agriculture Department:

Researcher
Agriculture

In this position, you will investigate the best systems for protected agriculture under arid Kuwait conditions.

We require a PhD in Agricultural Engineering, or a related discipline, and a minimum of 4 years' experience in greenhouse system design, operation, productivity and evaluation.

Researcher
Poultry Nutrition

Working to investigate the variables affecting poultry nutrition and management in Kuwait, you will design nutritional management studies of various housing systems, using a minicomputer to obtain the lowest cost feed formulation and analysis of experimental data.

We require an M.SC. in Poultry Nutrition and Physiology and 5 or more years' research experience.

Kuwait Institute offers attractive salaries commensurate with qualifications and experience, furnished housing, liberal fringe benefits including round trip air tickets and free medical benefits.

Please airmail your complete resume by **October 24, 1979** to: **Mr. Habib Al-Sahhaf, Personnel Manager, Kuwait Institute for Scientific Research, P.O. Box 24885, Safat, Kuwait, State of Kuwait.**

Kuwait Institute for Scientific Research

W360(A)

ST GEORGE'S HOSPITAL MEDICAL SCHOOL
(University of London)

TEACHING TECHNICIANS

Grades AB and 3 required to assist the Superintending Technician in the preparation of histology sets and anatomical specimens to support teaching commitments in the Structural Biology Department. Applicants should have at least three years experience in a Biological laboratory. Further training to gain higher technical qualifications will be encouraged.

Grade 2b range £2,937 — £3,546. Grade 3 range £3,122 — £3,770. London allowance £275 p.a. Salaries and London allowance are under review.

Application forms and further particulars obtainable from the Establishment Officer, St George's Hospital Medical School, Cranmer Terrace, London, SW17 0RE. For further details prospective candidates are invited to contact Mr J T Fish, Superintending Technician, 01-672 1255 Ext. 4615.

3267(A)

UNIVERSITY OF EXETER
DEPARTMENT OF GEOGRAPHY

TECHNICIAN
Grade 3

Applications are invited for the post of

GEOGRAPHY TECHNICIAN,

to work in the physical geography laboratories. The work involves mainly physical and some chemical analysis of soils and unconsolidated sediments.

Applicants should possess the Science Laboratory Technician's Part I Certificate or equivalent.

Salary scale £3,122 to £3,553 p.a. (becoming £3,313 to £3,770 p.a. at 1.4.80), (under review), with initial placement according to age, qualifications and experience.

Further particulars from Miss Doreen Watson, Northcote House, Queens Drive, Exeter, EX4 4QJ, to whom applications should be sent by 7 October 1979. Please quote reference No. 5130.

3230(A)

ELECTRON MICROSCOPY UNIT
Institute of Dermatology London E9

(University of London)

A post-doctoral research assistant is required to work on a project on cutaneous vascular responses to injury. The appointment will be for two years in the first instance with possibility of extension for a further year. Experience in E.M. techniques is essential. Starting salary £4,333 to £4,910 plus L.W. (£502) p.a.

Address applications, including full C.V. and names of two referees, or requests for further information, to Dr R A J Eady, Institute of Dermatology, St John's Hospital for Diseases of the Skin, Homerton Grove, London E9. Tel: 01-985 7061. Closing date is October 19th, 1979. 3268(A)

NEUROLOGICAL RESEARCHER
ASSOCIATE PROFESSOR LEVEL

The Virginia Mason Research Center is establishing a new Neuroscience Research Laboratory and would require the services of an experienced investigator in some area of neuroscience who could actively collaborate with clinicians in the Neurology and Neurosurgery Departments of the Mason Clinic and Virginia Mason Hospital. It is expected that this person will be capable of obtaining research grants nationally which, together with continued bequests, will allow the laboratory to grow, expand and develop. The VMRC is a $2,000,000 a year research center which houses the activities of 11 Senior Scientists, 20 Research Associates, and approximately 40 technicians and other professionally trained assistants.

Please send resumes to John C. Houck, PhD, Director, Virginia Mason Research Center, 1000 Seneca Street, Seattle, Washington 98101.

Virginia Mason Research Center is an Equal Opportunity Employer. W357(A)

THE ROWETT RESEARCH INSTITUTE
Bucksburn, Aberdeen,
AB2 9SB

ANALYST
(Two year appointment)

An Analyst is required in the Applied Nutrition Department to undertake studies of the products arising from the large-scale fractionation of grass and other fodder crops. Candidates should have had training in chemical analytical techniques. Appointment will be for a period of 2 years.

Qualifications required: A degree in Chemistry or Biochemistry, or Higher National Certificate in Chemistry with relevant experience.

Appointment will be in the Scientific Officer or Higher Scientific Officer grade. Salary scale £3,289 to £5,085 (S.O.) or £4,727 to £6,211 according to qualifications and experience. On 1 January 1980 these scales will be increased to S.O. £3,591 to £5,486 and H.S.O. £5,097 to £6,737. At least 5 years' relevant post qualifying experience is normally requierd for H.S.O. grading.

Non-contributory superannuation scheme.

Further particulars are available. Applications, including the names and addresses of 2 referees, should be submitted to the Secretary of the Institute by 12 October 1979. 3223(A)

UNIVERSITY OF YORK MICROTEST

A young and energetic

RESEARCH SCIENTIST

is required by the above contract research group. The person appointed would be responsible for the day-to-day running of a large project concerned with determining the biological activity of diesel and petrol engine exhausts. Candidates should have a strong background in chemical fractionation procedures as well as an interest in short-term tests for carcinogens/mutagens. Starting salary up to £8,182 pa (under review).

Informal enquiries can be made by contacting Dr R.C. Garner, Cancer Research Unit (0904 411805). Four copies of applications and curricula vitae, naming three referees, should be sent by Monday 1 October 1979 to the Registrar, University of York, Heslington, York, YO1 5DD. Please quote reference numbr 11/6112.

3261(A)

UNIVERSITY OF BRISTOL
DEPARTMENT OF BIOCHEMISTRY

Applications are invited from biochemists or microbiologists for the post of

SRC POSTDOCTORAL RESEARCH ASSISTANT

to study the means by which soil bacteria obtain energy for growth from the oxidation of ammonium ions. Previous experience in microbiology is desirable. The appointment is for three years with a salary up to the rate of £4,910 per annum. (Scale effective from 1st October, 1979 and subject to further review).

Applictions, including a brief curriculum vitae and the names of two referees should be sent by 4th October 1979 to Dr P.M. Wood, Department o Biochemistry, Medical School, University of Bristol, University Walk, Bristo BS8 1TD. 3241(A)

UNIVERSITY OF OTAGO
Wellington Cancer and Medical Research Institute

Wellington Clinical School of Medicine Wellington, New Zealand

RESEARCH OFFICER
(Medical or Non-Medical)

Applications are invited from medical o science graduates for the position o Research Officer. Science graduates should possess a higher qualification such as PhD Medical graduates if not in possession of higher degree, will be encouraged to enro for such a qualification. Previous researc experience is not essential. The appointe will work in the field of atherogenesis an experimental pathology under the directorship of Professor W.E. Stehbens Commencing salary according t qualifications and experience within th scale NZ$14,673 to $25,729 (Medical) NZ$11,894 to $18,665 (Science graduates) Increases are pending.

Further particulars are available fror the Association of Commonwealt Universities (Appts), 36 Gordon Square London WC1H 0PF, from the Registrar c the University, P.O. Box 56, Dunedir New Zealand, or from the Director of th Institute (Professor W.E. Stehbens Wellington Clinical School, Wellingto Hospital, Wellington, New Zealand.

Applications close on *31 October 1979*

3233(A)

FACULTY, HUMAN BIOLOGY PROGRAM at Stanford University

in field of Public Policy Analysis. Half-time in Human Biology, a large undergraduate program integrating biological and behavioral sciences. Half-time in related Stanford department or school. Person with demonstrated research productivity, interested in undergraduate teaching, with organizational skills to help teach and coordinate a series of courses in health policy and environmental policy. Rank open. Begin September 1, 1980. Affirmative Action Employer. Application deadline October 15, 1979. Send vita to Albert H. Hastorf, Policy Search Committee, Human Biology, Stanford University, Stanford, CA 94305. W368(A)

UNIVERSITY OF ESSEX

FELLOW

in the

DEPARTMENT OF PHYSICS

(Laser-Photoconductivity in Semiconductors)

Applications are invited for a post of Fellow (starting salary up to £4,910 on Range 1A) tenable for 3 years, to carry out S.R.C. — funded experimental research into deep-level impurities in semiconductors in collaboration with Dr B.K. Ridley. Candidates should have a PhD or equivalent qualification. Experience of infrared lasers, semiconductors and low-temperature techniques would be useful but not essential.

Application should be made to Mrs D.A. Weatherall, Department of Physics, University of Essex, Wivenhoe Park, Colchester CO4 3SQ (tel. 0206 862286). 3270(A)

COMMONWEALTH AGRICULTURAL BUREAUX

Vacancy for

SCIENTIFIC INFORMATION OFFICER

at the

Commonwealth Bureau of Horticulture and Plantation Crops East Malling Research Station, Nr. Maidstone, Kent

Duties: Preparing abstracts in English for the journal *Horticultural Abstracts*, which covers world scientific literature in this field, indexing and answering scientific enquiries.

Qualifications: A degree in horticulture, agriculture, botany, or related subject, plus a reading knowledge of at least one European language (German preferred) and the ability to write good English. Applicants must be able to render the content of scientific publications into concise, accurate and readable abstracts. It is expected that the successful candidate will wish to make a career in the field of scientific literature rather than research.

Salary: In the scale £2,839 to £5,448 (currently under review), plus a compensatory allowance (taxable but not superannuable) of 3.3 per cent of basic salary to offset personal contribution to superannuation scheme. Starting salary according to qualifications and experience. Promotion to higher scales on merit.

Application forms and full particulars from the Executive Director, Commonwealth Agricultural Bureaux, Farnham House, Farnham Royal, Slough SL2 3BN.

Closing date for applications: 10 October 1979. 3222(A)

DIRECTOR HUMAN BIOLOGY PROGRAM at Stanford University.

Person with demonstrated research productivity, interested in undergraduate teaching, with organizational skills to administer large program integrating biological and behavioral sciences, with some policy emphasis. Half-time in Human Biology Program, half-time in a biologically related department in which applicant's experience merits tenure appointment as associate or full professor. Begin appointment September 1, 1980. Affirmative Action Employer. Application deadline October 15, 1979. Send vita to Sanford M. Dornsbusch, Human Biology, Stanford University, Stanford, CA 94305. W367(A)

UNIVERSITY OF EDINBURGH MEDICAL SCHOOL

DEPARTMENT OF MEDICINE

Applications are invited for a

POSTDOCTORAL RESEARCH FELLOW

to work with CRC funded project on immune complexes in disease. Post tenable until September 1980 in the first instance. Salary scale £4,333 to £7,521 per annum according to qualifications.

For further particulars write to Dr W.J. Irvine, Endocrine Unit and Immunology Laboratories (Medicine), The Royal Infirmary, Edinburgh. Applications quoting the names of two referees should be addressed to the Secretary to the University, University of Edinburgh, Old College, South Bridge, Edinburgh, EH8 9YL not later than 11th October 1979. Please quote Reference 5060. 3255(A)

UNIVERSITY OF THE WITWATERSRAND, Johannesburg, South Africa

DEPARTMENT OF MICROBIOLOGY

LECTURER

Applications are invited from suitably qualified persons for appointment to the above post.

Applicants could have an interest in any aspect of Microbiology, although those with experience in bacteriological techniques as applied to ecology or plant pathology are encouraged to apply.

In addition to pursuing his/her own research, the incumbent will be required to participate in the teaching of the undergraduate and postgraduate courses given to students in the Faculty of Science, and to supervise research students.

Duties are to be assumed as soon as possible. The salary range is R8 100 to R13 100 per annum, the initial salary being determined according to qualifications and experience. Benefits include an annual bonus, pension and medical aid facilities and a housing subsidy, if applicant is eligible. (£1 = R1.87 approx).

The University's policy is not to discriminate in the appointment of staff or the selection of students on the grounds of sex, race, religion or colour. Further particulars relating to this practice and to the above post are included in an information sheet obtainable from the London Representatve, University of the Witwatersrand, Chichester House, 278 High Holborn, London WC1V 7HE, or from the Registrar, University of the Witwatersrand, Jan Smuts Avenue, Johannesburg, South Africa, with whom applications should be lodged by 15th October 1979. 3232(A)

We have the following vacancies in our Biochemistry Section:

Experimental Officer — Biochemist

A graduate biochemist to play a part in the discovery of new insecticides. An understanding of neurobiology would be desirable, but practical experience in insect biochemistry is not essential. However, candidates must have considerable experience of modern biochemical techniques.

The successful applicant will join a small team composed of one PhD and one graduate, responsible for initiating research in areas of insect biochemistry that could lead to a programme of chemical synthesis or produce *in vitro* methods for detecting active chemicals. Complementary work will be concerned with evaluating the mode of action of promising new insecticides and gaining an early understanding of the factors that may contribute to the development of resistance.

Experimental Officer — Physical Chemist/ Electrochemist

A Physical Chemist to work on the electrochemistry and reaction kinetics of exploratory organic compounds being synthesised as potential pesticides. A knowledge of chemistry at least up to HNC level is required, and experience of polarography or related techniques is desirable but not essential. The successful candidate will work closely with organic and bio-chemists, and will be in a small team headed by a PhD physical chemist. There will be scope for initiative in developing techniques and helping to design structures with the desired electrochemical properties.

Jealott's Hill is the chemical and biological research centre for the crop protection interests of ICI. It is situated in open country 30 miles west of London, between Bracknell and Maidenhead. We offer competitive salaries and fringe benefits including membership of the ICI Profit Sharing and Pension Fund. Coaches run between Jealott's Hill, Bracknell and Maidenhead.

If you are interested in this vacancy please write or telephone for an application to:

Mr S R Stephenson
ICI Plant Protection Division
Jealott's Hill Research Station
Bracknell, Berks. *Ref no 79/Biochem*

ICI Plant Protection Division

3231(A)

POSTDOCTORAL FELLOW

Post available immediately for PDF to work on gas/solid reactions. Stipend and benefits up to maximum allowable by NSERC regulations.

Please forward applications with names of two referees to:

Dr R.A. Ross
Department of Chemistry
Lakehead University
Thunder Bay
Ontario
Canada P7B 5E1

W365(A)

Lakehead University

UNIVERSITY OF SHEFFIELD

A vacancy exists in the Department of Medical Microbiology at the Medical School, Hallamshire Hospital for a

MEDICAL LABORATORY SCIENTIFIC OFFICER

to take part in a 3 year research programme concerned mainly with immunological technique as applied to aspects of cancer research and other clinical problems. The post affords an opportunity for a suitable candidate to prepare a thesis for a higher qualification. Enquiries regarding the post to Dr A. Clark, telephone number (0742) 26484, ext. 2968/2027. Salary on N.H.S. scale for M.L.S.O.'s £3,768 to £5,391 p.a. (under review). Please write, giving the names of two referees to the Administrative Officer (Personnel), Ref. S 1374/N, The University, Sheffield S10 2TN. 3257(A)

Ministry of Agriculture, Fisheries and Food

Scientist

to help co-ordinate R & D

. . . to join the Chief Scientist's Group which is responsible for the planning of the research and development programme for agriculture, fisheries and food.

The Scientist appointed will help to liaise with food manufacturers and contractors and review annually the commissioning and financing of R & D activities in the food industry. The work includes classifying current food R & D activity, drafting reports and papers, reviewing technical and scientific literature and interpreting the data.

No laboratory work is involved but some visits to R & D establishments may be required.

Candidates (normally aged under 27) should have a good honours degree or equivalent in a suitable scientific subject.

Appointment will be as Scientific Officer £4,065 to £5,865 (to become £4,370 to £6,265 from 1.1.80). Promotion prospects. Non-contributory pension scheme.

For further details and an application form (to be returned by 15th October, 1979) write to Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, or telephone Basingstoke (0256) 68551 (answering service operates outside office hours). *Please quote reference SB/43/AE.* 3226(A)

Science Group
CIVIL SERVICE

VIROLOGIST
Assistant Professor

Two-year appointment with the possibility of tenure track. Salary commensurate with experience. To teach 2 half year lecture courses in Virology at the third and fourth year levels plus 1 half year Virology techniques laboratory. The candidate should have a PhD, some post-doctoral experience and be capable of developing a research program in Virology.

Excellent research facilities including Tissue Culture, Radioisotope and Biohazard containment laboratories are available.

The position is to start January 1, 1980, or as soon as a suitable candidate can be found.

Curriculum Vitae with names of 3 referees should be sent not later than December 15, 1979 to:

Chairman,
Department of Biology,
University of New Brunswick,
Fredericton, N.B.,
Canada E3B 5A3

W371(A)

THE QUEEN'S UNIVERSITY OF BELFAST
DEPARTMENT OF AGRICULTURAL AND FOOD CHEMISTRY

POST-DOCTORAL RESEARCH ASSISTANT

Applications are invited from plant physiologists for an ARC sponsored research project investigating the genetic control of photorespiration in ryegrass and other crop plants. The project will be under the direction of Dr M.K. Garrett and aims to investigate the occurrence of RuBP carboxylase/oxygenase variants and interpret their physiological significance. Experience in enzymology and whole plant physiology is essential. Appointment is for 3 years with a starting salary in the range £4,333 to £4,910 with USS. Applicants should submit a full curriculum vitae including the names and addresses of two referees to the Personnel Officer, The Queen's University of Belfast, Belfast BT7 1NN, Northern Ireland. Closing date: 26th October 1979. 3219(A)

QUEEN ELIZABETH HOSPITAL FOR CHILDREN
DEPARTMENT OF CYTOGENETICS

Hackney Road, London E2 8PS

BASIC GRADE CYTOGENETICIST

Applications are invited from honours graduates whose biological science courses contained units in genetics/cytogenetics, cell biology and biochemistry. Some knowledge of data processing would be an advantage.

Employment will be on the Basic Grade PTA scale for graduates in the National Health Service in accordance with Whitley Council Regulations. Current starting salary £4,035 p.a. + £354 London Weighting p.a.

Apply by letter to the Principal Cytogeneticist enclosing a curriculum vitae and the name of two referees.

Closing date 4th October, 1979. 3214(A)

HARVARD UNIVERSITY

PROFESSOR
HEALTH POLICY ANALYSIS
DEPARTMENT OF BIOSTATISTICS

The position will require an extensive background, and demonstrated research productivity in the application of quantitative analytical techniques to programmatic evaluation and of decision analytic concepts to policy issues in the health sector. The investigative activities of the faculty member will involve collaborative research with physicians and other members of the health-related professions as well as the teaching of statistical and other quantitative analytic methods to graduate students, and supervision of doctoral candidates.

Send application and vitae to Frederick Mosteller, Chairman, Department of Biostatistics, 7th Floor, School of Public Health, Harvard university, 677 Huntington Avenue, Boston, MA 02115.

Harvard University is an Equal Opportunity/Affirmative Action Employer. W374(A)

AGRICULTURAL RESEARCH COUNCIL
Food Research Institute
SCIENTIFIC SERVICES AND DEVELOPMENT DIVISION

CHEMIST

A Scientific Officer is required to join, on a temporary basis, the Chromatography Development Group which is part of the Scientific Services and Development Division.

The appointment relates to a research contract ending in October 1980, which has as its objective the estimation in poultry waste of residues of specified veterinary products.

Published gas chromatographic methods will be worked up but some method development will be necessary.

A pass degree or HNC in chemistry, together with a knowledge and practical experience of high performance liquid chromatography and/or gas chromatography, are required.

Salary: From 1st August 1979: on a scale £3,289 to £5,085. From 1st January 1980: on a scale £3,591 to £5,486.

Five-day working week and flexible working hours scheme operated. Transport facility available mornings and evenings.

Further particulars and application forms from: The Secretary, Food Research Institute, Colney Lane, Norwich NR4 7UA quoting Ref. No 79/9. Closing date 4th October, 1979. 3265(A)

MRC PNEUMOCONIOSIS UNIT
Llandough Hospital, Penarth, South Glam. CF6 1XW.

POST-DOCTORAL RESEARCH SCIENTIST

Applications are invited for a three year post-doctoral appointment at the above Unit. The successful applicant will join the cellular immunology group, studying the effects of dust exposure on human populations and on the cells of the immune system of animals. He/she will be involved in the development of techniques related to human monocytes and rat macrophages associated with tumours produced by dusts.

The salary will depend on experience and will be within the range £4,232 to £6,627.

For further details and an application form write to the Assistant Administrative Officer. 3259(A)

THE LONDON HOSPITAL MEDICAL COLLEGE
(University of London)
BONE AND JOINT RESEARCH UNIT

RESEARCH ASSISTANT

Applications are invited from graduates with a good honours degree in physiology, biophysics or similar qualifications, for the appointment of a Research Assistant within a team studying the physical chemistry of cartilaginous tissues. The successful candidate will be required to investigate the effect of mechanical loading on the permeability and structure of the tissue matrix.

This appointment is for a period of three years and is financed by a grant from The Wellcome Trust. Initial salary, inclusive of London Allowance, in the region of £5,000 per annum (subject to review as from 1st October 1979).

Further enquiries to Dr Maroudas, Tel. 01-247-5454 Ext. 421. Applications to The Secretary, The London Hospital Medical College, Turner Street, London E1 2AD. 3260(A)

British Nutrition Foundation

The BNF combines the expertise of leading medical, academic, governmental and industrial experts in its educational and research activities.

There are two vacancies for persons of high ability:

SENIOR SCIENTIFIC OFFICER (SSO)
SCIENTIFIC INFORMATION OFFICER (SIO)

The SSO will have a good honours degree and several years relevant post-graduate experience. The varied work will include 'in depth' studies and the preparation of reports for publication.

The SIO will have a good honours degree or other relevant qualification and experience, and must be capable of explaining matters relating to food, nutrition and health in simple terms, by written and spoken word to health and education professionals and to lay persons.

Applications should reach the Secretary, British Nutrition Foundation, 15 Belgrave Square, London SW1, before Monday 1 October 1979.

3264(A)

UNIVERSITY OF NOTTINGHAM MEDICAL SCHOOL

DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY

POSTGRADUATE/POST-DOCTORAL RESEARCH ASSISTANT IN NEUROPHARMACOLOGY

Applications are invited for this post, funded by The Wellcome Trust, to work with Dr C. A. Marsden on the effects of long-term antidepressant drug administration on neurotransmitter systems in the rat using *in vivo* electrochemical techniques. Previous experience with implanting eletrodes in the CNS would be an advantage. The appointment is for three years commencing October 1979 or as soon as possible thereafter. Initial salary in the range of £3,775 to £4,333.

Applicants should send their curriculum vitae and names of two academic referees to Professor P.H. Fentem, Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH as soon as possible.

3234(A)

THE UNIVERSITY OF LEEDS

DEPARTMENT OF BIOCHEMISTRY

Applications are invited for a temporary post of

RESEARCH FELLOW

in the above Department to work with Dr D.A. Harris on a project supported by the S.R.C. on enzymology of ATP synthesis by mitochondria and/or Chloroplasts. The work will involve studies of chemical modification of isolated and membrane proteins, and rapid reaction kinetics. Candidates should already possess, or expect to be awarded, a PhD degree, within the next few months. The appointment will be made for a fixed period of three years.

Starting salary in the range £4,333 to £4,910 on the 1A scale for Research and Analogous Staff (£4,333 to £7,521) (under review).

Informal enquiries about the post will be welcomed by Dr D.A. Harris (Telephone (0532) 36171 ext. 86).

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference number 83/24/D. Closing date for applications 12 October 1979.

3220(A)

USER SUPPORT PROGRAMMER

The Daresbury Laboratory, located in the Cheshire countryside, is developing major national facilities for research into atomic, molecular, solid state and nuclear physics. Exploitation of these facilities requires first-class computing support. Computers at the Laboratory include a CRAY-1, an IBM 370/165, GEC 4070's, PDP-11's and Interdatas.

The vacancy is in the user support group which is responsible for producing general support for users of the Daresbury computer system. An important facet of this work is the exploitation of the powerful CRAY-1 computer which is to be used for extremely large scientific calculations. Computing experience, especially scientific computation, is required although training will be provided.

Appointment will be made at Higher Scientific Officer grade. Applicants, of either sex, should possess a good honours degree in a relevant subject, plus at least 2 years post graduate experience.

Salary will be assessed according to experience on the following scale:
£4,727 to £6,211 which will increase from 1st January 1980 as follows:
£5,097 to £6,737

There is a non-contributory superannuation scheme, a generous leave allowance and a flexible working hours scheme.

Closing date 19th October 1979.

Please write, or telephone Warrington 65000 Ext. 467 for an application form quoting reference No. DL/703 to

The Personnel Officer
DARESBURY LABORATORY
Science Research Council
Daresbury, Warrington WA4 4AD

3269(A)

UNIVERSITY OF YORK

DEPARTMENT OF BIOLOGY

RESEARCH ASSISTANT

Applications are invited for a Research Assistantship for three years funded by the Nuffield Foundation to work on the cell-free synthesis of myelin proteins. Salary on the IB scale. Applicants should be of graduate standard with a qualification in Biology, Physiology or Molecular Biology.

Further information is available from Dr S J S Hardy or Dr M G Rumsby at the address below. Three copies of applications and curricula vitae, naming two referees should be sent as soon as possible to the Registrar, University of York, Heslington, York YO1 5DD. Please quote reference number 11/7082.

3242(A)

UNIVERSITY COLLEGE CARDIFF

Applications are invited for the

CHAIR IN MINERAL EXPLOITATION.

The salary will be within the Professorial range. Duties to commence on 1st January 1980, or as soon as possible thereafter.

Applications (two copies), together with the names and addresses of three referees should be forwarded, no later than the 31st October, 1979, to the Vice-Principal (Administration) and Registrar, University College, P.O. Box 78, Cardiff CF1 1XL from whom further particulars may be obtained. Reference 1897.

3254(A)

DEPARTMENT OF CHILD HEALTH

UNIVERSITY OF MANCHESTER

RESEARCH FELLOW IN ANATOMY

Postdoctoral Research Fellow required in multidisciplinary research team to organise new work in quantitative histology, mainly but not exclusively in the brain.

Enquiries with full curriculum vitae to Professor John Dobbing, The Medical School, Oxford Road, Manchester M13 9PT

3197(A)

UMIST

DEMONSTRATOR IN MICROSCOPY AND PHYSIOLOGY

Applicants should have a recent PhD or MSc. The post includes both research and teaching. Research work will be in collaboration with Dr N M Tyrer into synaptic structure and function in the insect CNS, involving electron microscopy of cobalt filled neurones and histochemical identification of synaptic transmitters. Teaching duties include supervision of third year EM projects and demonstrating in an elementary physiology course.

Salary is on the scale £3,775 to £5,488 per annum.

Letters of application, quoting reference B10/179, including a curriculum vitae and the names of two referees, should be addressed to Dr N M Tyrer, Department of Biochemistry, UMIST, PO Box 88, Manchester, M60 1QD.

3249(A)

X-RAY CRYSTALLOGRAPHER Research Associate to conduct x-ray diffraction studies on liquid crystals in well equipped laboratory. Required PhD or equivalent experience. Salary: $11-15,000 annually, depending on qualifications. Reply to Dr Leonid V Azaroff, Director, Institute of Materials Science, University of Connecticut, Storrs, CT 06268 before October 22, 1979. An Equal Opportunity Employer.

XW328(A)

UNIVERSITY OF NATAL

DEPARTMENT OF GEOLOGY

PIETERMARITZBURG

Applications are invited from suitably qualified persons regardless of sex, religion, race, colour or national origin, for appointment to the post of

LECTURER

The salary will be in the range:

R8 100 — R13 200 per annum

The commencing salary will be dependent on the qualifications and/or experience of the successful applicant. In addition, an annual vacation savings bonus is payable, subject to Treasury regulations.

Application forms, further particulars of the post and information on pension, medical aid, group insurance, staff bursary, housing loan and subsidy schemes, long leave conditions, and travelling expenses on first appointment are obtainable from the Registrar, University of Natal, P.O. Box 375, Pietermaritzburg, 3200, South Africa, with whom applications, on the prescribed form, must be lodged not later than 9 November 1979, *quoting reference PMB 62/79.*

W363(A)

Upjohn *medicine . . . designed for health . . . produced with care*

GRADUATE

STRERILE PHARMACEUTICAL PRODUCTION

Upjohn Limited is the wholly owned subsidiary of The Upjohn Company, USA, and is a leading pharmaceutical house based in West Sussex. A vacancy has arisen for a graduate to join our production team in the Sterile Products Manufacturing Unit, formulating and aseptically packaging a variety of sterile ethical human and veterinary products.

The successful applicant will eventually be responsible for either the manufacturing and preparation operations, or the aseptic filling operations on a rotational basis. This will involve supervising and controlling the work of ten to fifteen operatives at any one time.

Applications are invited from graduates in Microbiology, or who are M.I.Biol. qualified, and who have up to two years experience in the pharmaceutical or similar industry.

In return we can offer a competitive compensation package which includes a 36 ¼ hour working week, contributory pension scheme, free life assurance and disability plans and relocation assistance where appropriate.

For further information and an application form candidates should apply to:-

The Personnel Officer,
Upjohn Limited,
Fleming Way,
Crawley,
West Sussex RH10 2NJ
Telephone: Crawley 31133.

3258(A)

INSTITUTE OF CANCER RESEARCH: PHYSICIST required for interesting post in Radiological Physics at the Royal Marsden Hospital, Fulham Road, London, SW3. The person appointed will be one of a team covering radiotherapy, diagnostic radiology, and nuclear medicine. He or she will have special responsibilities for Radiation Protection and for new techniques in diagnostic radiology. Some postgraduate experience in this or a related field is desirable but new graduates with good degrees may apply. Appointment at an appropriate point on Grade II of MRC scales £4,261 p.a. to £6,627 p.a. plus London Allowance of £502 p.a. Apply in duplicate giving full CV and names of two referees to the Secretary, Institute of Cancer Research, 34 Sumner Place, London, SW7 3NU, quoting ref. 300/G/15. 3228(A)

FACULTY POSITIONS.

THE DEPARTMENT OF CHEMISTRY

SYRACUSE UNIVERSITY

invites applications from outstanding candidates in all fields of chemistry. During the coming year we expect to make two or three appointments, probably at the level of assistant professor, tenure track, with possibility of appointment at chemistry, organometallic chemistry, and analytical chemistry.

Applicants should send a complete curriculum vitae, list of publications, summary of teaching experience, and a statement of research plans, and should arrange for at least three letters of recommendation to be forwarded to support the application.

Nominations and applications should be mailed by November 1 to Professor B.R. Ware, Chairman, Department of Chemistry, Syracuse University, Syracuse, New York 13210. Syracuse University is an Equal Opportunity/Affirmative Action employer. W369(A)

UNIVERSITY OF BRISTOL

SCHOOL OF CHEMISTRY

GRADUATE RESEARCH ASSISTANT

Applications are invited from plant biochemists or plant biologists for this post to undertake research into the identification, photo-control of levels, and metabolism of gibberellins in plant leaves and etioplasts. The post which is funded by ARC is tenable for three years at a salary of £3,775 to £4,333 (Scale effective from 1st October, 1979 and subject to further review). The successful candidate will be encouraged to register for a higher degree.

Applicants should send a curriculum vitae and names of referees as soon as possible, to Professor J. MacMillan, School of Chemistry, The University, Bristol BS8 1TS. 3164(A)

KING'S COLLEGE LONDON

RESEARCH TECHNICIAN

Applications are invited for this post to assist with a project investigating the properties of pyruvate carboxylase in wild-types and mutant strains of Aspergillus nidulans. A knowledge of enzymology, microbiology and associated biochemical techniques is desirable. Minimum qualifications, ONC/HNC or equivalent, preferably with several years relevant experience. This post is financed by the SRC for 1 year with the possibility of renewal for a further 1 year. Salary on scale £3,646 pa to £4,077 pa inclusive (under review). Five weeks annual holiday.

Interest-free loans for annual rail season tickets.

Apply in writing with full details, naming two referees to: The Head Clerk (Ref: 221707/N), King's College London, Strand, WC2R 2LS. 3253(A)

LEICESTER POLYTECHNIC

SCHOOL OF CHEMISTRY

SENIOR TECHNICIAN IN MASS SPECTROMETRY

Applications are invited from graduates in a relevant science for this post which will involve operating a new mass spectrometer system comprising a PYE 204 Chromatograph, a VG 16 F Mass Spectrometer and a VG Display Digispec Data System.

In addition to providing a service for the teaching and post graduate work of the Science Faculty there will be novel and interesting duties in connection with the provision of a service to industrial users on a commercial basis; this will involve extensive liaison with industrial laboratories.

Applicants should have at least three years experience as mass spectroscopists and preferably be competent in the maintenance of the system.

Salary: £5,220 to £5,547 per annum.

Application forms available from Staffing Officer, Leicester Polytechnic, PO Box 143, Leicester LE1 9BH. Tel (0533) 551551 ext 2303. 3238(A)

STANFORD UNIVERSITY

GEOCHEMIST —PETROLOGIST

The Department of Geology in the School of Earth Sciences invites applications for a faculty position beginning September, 1980. Areas of interest include (but are not limited to) geochemical processes in the crust and mantle of the earth including isotope geochemistry, thermodynamics, kinetics, element distribution and phase equilibria studies. Teaching responsibilities might include graduate and undergraduate courses in geochemistry and igneous petrology. Level of appointment is open, but we are particularly interested in a creative young person, with a well-established record in research.

Applications should include (1) a resume of education, professional experience, and published research; (2) a brief statement of plans for future research, and (3) the names of 4 referees. Send to: Prof. J.G. Liou, Department of Geology, Stanford University, Stanford, CA 94305, by December 1st, 1979. Stanford University is an equal-opportunity/affirmative-action employer. W329(A)

UNIVERSITY OF EDINBURGH:

THE EDINBURGH SCHOOL OF AGRICULTURE

POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited from plant pathologists, soil microbiologists or those in related disciplines, for a 3 year post-doctoral position financed by the ARC from 1st October 1979. The study involves early stages of parasitism by cereal root- and foot-rot fungi; it is hoped to determine if natural senescence of host tissues enhances infection and if biological control operates by competition for senescing tissues. This has important fundamental and practical implications.

Starting salary £4,522, rising to £4,865 in third year.

Applications by *curriculum vitae*, with names of two referees, to Dr J.W. Deacon, Microbiology Department, School of Agriculture, West Mains Road, Edinburgh, EH9 3JG, from whom further particulars can be obtained. 3212(A)

INSTITUTE OF CANCER RESEARCH: TECHNICIAN required for Treatment Planning Group, Physics Department, Royal Marsden Hospital, Fulham Road. Applicants should have a degree in Physics or appropriate experience in radiological physics. The appointed person will be trained to prepare treatment plans using a PDP8E computer and will be closely involved with radiotherapy techniques and measurements. Treatment machines include three Cobalt units, at 10 Mv Linear Accelerator and orthovoltage X-ray equipment. Appointment will be at the appropriate point on the Technician scale £3,261 p.a. to £4,680 p.a. (scales under review) plus London Allowance of £354 p.a. Apply in duplicate giving the names of two referees to the Secretary, Institute of Cancer Research, 34 Sumner Place, London, SW7 3NU, quoting ref. 301/B/85. 3227(A)

ASSISTANT PROFESSOR OF MICROBIOLOGY. The Department of Microbiology and Immunology, Washington University School of Medicine invites applications from highly qualified individuals for an appointment as Assistant Professor. Applicants should have PhD or MD degrees, 2-5 years of post-doctoral research experience and a commitment to medical and graduate student teaching. Teaching competence should be in pathogenic microbiology; research interests should be in microbiology. Send curriculum vitae, brief description of research plans and names of three referees to Dr Joseph M Davie, Department of Microbiology and Immunology, Washington University School of Medicine, St Louis MO 63110 by November 1, 1979. Equal Opportunity and Affirmative Action Employer M/F/H. W339(A)

MEDICAL RESEARCH COUNCIL

LABORATORY OF MOLECULAR BIOLOGY

POST-DOCTORAL RESEARCH SCIENTIST

is required for a short-term three-year appointment.

The person appointed will be required to work at the Institute of Animal Physiology at Babraham, Cambridge, and will work on the subject of complement activation and its application to clinical problems.

Salary on scale starting from £5,048 (pay award pending).

Applications, with *c. v.* and names of two referees, should be sent to the Administrative Secretary, MRC Laboratory of Molecular Biology, The Medical School, Hills Road, Cambridge CB2 2QH, to be received not later than 1st November, 1979. 3277(A)

UNIVERSITY OF DURHAM

DEPARTMENT OF PHYSICS

Applications are invited for a

POSTDOCTORAL SENIOR RESEARCH ASSISTANT

tenable for three years and three months from 1 November 1979 to work with Professor B.H. Bransden in the Department of Physics on the Theory of Atomic Charge-Exchange Reaction, with special reference to Heavy Ion Fusion.

Initial salary in the range £4,333 to £7,227 on National Range 1A plus superannuation.

Applications (3 copies) naming three referees should be sent by 19 October 1979 to the Registrar and Secretary, Science Laboratories, South Road, Durham, DH1 3LE from whom further particulars may be obtained. 3276(A)

UNIVERSITY OF BIRMINGHAM

FACULTY OF MEDICINE AND DENTISTRY

RESEARCH FELLOW GRADE Ia or II

equired to work in the Department of edicine (Clinical Oncology). The post volves studies on steroid and peptide ormone reseptors in Breast Cancer and ould be required to direct the research ork of a small Group of Fellows and echnicians and be responsible for onitoring routine steroide Hormone ssays.

Salary will be according to age and perience on the scale Research Fellow IA 4,333 to £7,521) or II (£6,932 to £8,992). ith super annuation benefits.

Further particulars may be obtained om professor R. Hoffenberg. epartment of Medicine Queen Elizabeth ospital Birmingham B152 TH.

Applications (Three Copies) should be nt to the assistant registrar, the Medical hool Birmingham B15 2TJ by 12th ctober 1979 please quote Ref.RF/Med 9. 3275(A)

THE UNIVERSITY OF SHEFFIELD

DEPARTMENT OF CHEMICAL ENGINEERING AND FUEL TECHNOLOGY

pplications are invited from men and omen for the post of

RESEARCH ASSISTANT

work with Professor J.A. Barnard on an R.C.-funded project entitled High emperature Pyrolysis of Hydrocarbons. he work will involve using a single-pulse ock tube to study the pyrolysis of ydrocarbons typical of those present in aphtha with the objective of establishing a odel of naphtha-cracking reactions at mperatures above those currently nployed in industry. Experience in gas netics and computing an advantage. itial salary up to £4,910 + U.S.S. Tenue years.

Applications to Professor J.A. Barnard, epartment of Chemical Engineering and uel Technology, the University, Sheffield 1 3JD, enclosing a curriculum vitae and ames of two referees. Quote ref: R363/G. 3237(A)

THE UNIVERSITY OF THE SOUTH PACIFIC — Fiji

Applications are invited for the post of

UNIVERSITY FELLOW (Post 79/84)

in the newly established Atoll Research Unit (Aru) of the Institute of Marine Resources (IMR). The Atoll Research Unit is to be located on Tarawa, in Kiribati. The appointee, who should be a marine biologist will be intially required to develop and carry out where practicable the applied research, advisory and educational programmes of the Unit, in consultation with the director of IMR. The most immediate project to be undertaken by the appointee is a survey of Tarawa lagoon and subsequent monitoring of any existing pollution indices. He/She will be appointed at lecturer or senior lecturer level. Housing and some laboratory facilities are available. An appropriate vehicle for transportation on Tarawa will be provided. Salary scales: Senior Lecturer — F$11960 to F$14135 pa, Lecturer — F$8175 to F$11563 pa (£1 sterling = F$1.83). The British Government may supplement salaries in range £3,504 to £4,662 pa (sterling) for married appointee and £1,788 to £2,664 pa (sterling) for single appointee (reviewed annually and normally free from tax) and provide children education allowances and holiday visit passages. Family passages; baggage allowance; 15% gratuity; superannuation scheme; appointment allowance; partly furnished accommodation at maximum rental of 12½% of salary (under review); 3 year contract.

Detailed applications (2 copies) with curriculum vitae and naming 3 referees to be sent direct to the Registrar, University of the South Pacific, PO Box 1168, Suva, Fiji by 24 October 1979. Applicants resident in the UK should also send one copy to Inter-University Council, 90-91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address. 3278(A)

UNIVERSITY COLLEGE LONDON

DEPARTMENT OF BIOPHYSICS

TECHNICIAN Grade 3

preferably with experience in tissue culture or histology to assist in nerve-muscle research. Appointment for one year with possibility of extension. Salary £3,205 + £524 London Weighting.

Applications to Professor R. Miledi, Dept. of Biophysics, University College London, Gower St, London WC1E 6BT. 3266(A)

THE AUSTRALIAN NATIONAL UNIVERSITY

Applications are invited from suitably qualified persons for appointment to the following positions:

JOHN CURTIN SCHOOL OF MEDICAL RESEARCH

Department of Pharmacology RESEARCH FELLOW

The successful applicant will be expected to pursue research under the general direction of Professor D.R. Curtis into synaptic mechanisms within the mammalian central nervous system at a cellular level, and to be familiar with the relevant anatomical, biophysical, and pharmacological techniques. Further information may be obtained from Professor Curtis, in the University. Closing date 15 October, 1979.

RESEARCH SCHOOL OF PHYSICAL SCIENCES

POSTDOCTORAL/RESEARCH FELLOWS:

In 1980 the Research School proposes to appoint up to six (School) Postdoctoral/Research Fellows, in addition to appointments to such positions falling vacant in specific departments.

The School currently consists of six departments (Applied Mathematics, Engineering Physics, Mathematics, Nuclear Physics, Solid State Physics and Theoretical Physics), three units (Diffusion Research, Electron and Ion Diffusion and Ultraviolet Physics), a Plasma Research Laboratory and the Mount Stromlo and Siding Spring Observatories; both the latter have the status of departments.

Nomenclature of appointment and salary will be determined in accordance with qualifications and experience. Closing date: 31 October, 1979.

DEPARTMENT OF THEORETICAL PHYSICS

POSTDOCTORAL FELLOW, RESEARCH FELLOW OR SENIOR RESEARCH FELLOW:

The Department would prefer to make the appointment in the research area of statistical mechanics, but applications from theoretical physicists with research interests in other areas will be considered. The Department's main current interests are in fundamental aspects of statistical mechanics, solid state physics, plasma physics, nuclear physics, elementary-particle physics and field theory. Applications would be welcomed from persons on leave from their institutions for shorter periods (but for at least two years for institutions outside Australia). Closing date: 30 November, 1979.

FACULTY OF ARTS

DEPARTMENT OF PREHISTORY AND ANTHROPOLOGY

Applications are invited for appointment to two posts in the Department of Prehistory and Anthropology. The headship of the Department alternates between Professor Anthony Forge and Professor D J Mulvaney.

LECTURER IN ANTHROPOLOGY:

Applicants should have a good scholarly record in research and teaching in Social or Cultural Anthropology. Experience in the field of Melanesia may be an advantage.

LECTURER IN BIOLOGICAL ANTHROPOLOGY:

Applicants should have research interests and teaching experience in biological anthropology which fit into the needs of the Department which has established streams of Prehistory and Social Anthropology and a developing program in Biological Anthropology. The successful applicant will have to teach in fields which aid the integration of these subjects, certainly including human adaptability, nutrition and growth, epidemiology, human variation and genetics.

There is already one Physical Anthropologist, Dr C.P. Groves, in the Department but other staff members of both streams are concerned with aspects of Biological Anthropology. Closing date 15 November, 1979.

Salaries: Salaries will be in accordance with qualifications and experience within the ranges: Senior Research Fellow $A22,755-27,143; Research Fellow $A16,291-21,265; Lecturer $A16,291-21,401; Postdoctoral Fellow — a fixed point within the range $A16,291-20,768. Present exchange rate $A1 = 50p. (A salary loading of up to $A2,500 per annum may be paid to an applicant for the position in Pharmacology holding appropriate medical qualifications).

Term of Appointment: Research Fellow/Senior Research Fellow: normally three to five years with the possibility of extension to a maximum of eight years. Research Fellow in Pharmacology — two years with possible extension to five years. Postdoctoral Fellow: one to two years, although persons from overseas are not normally appointed for less than two years. Lecturer: three years in the first instance with the possibility of reappointment after review to age 65.

Reasonable appointment expenses are paid. Superannuation benefits are available for applicants who are eligible to contribute. Assistance with finding accommodation is provided for an appointee from outside Canberra. The University reserves the right not to make an appointment or to make an appointment by invitation at any time.

Prospective applicants should first obtain the further particulars from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. 3213(A)

MARINE COORDINATOR

Person with the following qualifications: M.S. degree and/or five years experience in oceanography or a related field; must be thoroughly familiar with equipment, logistical problems and procedures used in marine sciences; must be able to do budgetary accounting and to supervise the work of a supporting staff. Salary range in $16-18,000 per annum. Application deadline is October 31, 1979.

Apply with résumé to:

Dr. William M. Sackett, Chairman
University of South Florida
Department of Marine Science
830 First Street South
St Petersburg, Florida 33701

W373(A)

TECHNICIAN/ RESEARCH OFFICER

required to assist in the Immunopathology Laboratory at our Mill Hill Laboratories, London, N.W.7. with investigations into relationships between autoimmunity malignancy and virus infection in mice.

HNC/Degree with experience in tissue culture techniques essential, particularly for the detection and isolation of RNA tumour viruses. Knowledge of basic immunology and embryology an advantage. Salary range £4,174 to £5,950.

For further information and application form, write or telephone to Miss S.M. Hurley, Imperial Cancer Research Fund, Lincoln's Inn Fields, W.C.2. on 242 0200 ext. 305, quoting reference 405/79. 3274(A)

THE UNIVERSITY OF PITTSBURGH SCHOOL OF MEDICINE

Pittsburgh, Pennsylvania

DEPARTMENT OF BIOCHEMISTRY CHAIRMAN

Applications and nominations are invited for the position of Chairman and William S. McEllroy Professor of the Department of Biochemistry. The candidates should be recognized scholars and investigators with demonstrated commitment to medical and graduate education.

Curriculum vitae and bibliography may be submitted to Professor Winton Tong, Department of Physiology, University of Pittsburgh, School of Medicine, Pittsburgh, Pennsylvania 15261.

An Equal Opportunity/Affirmative Action Employer. W362(A)

THE UNIVERSITY OF CALGARY

ARCTIC INSTITUTE OF NORTH AMERICA

EXECUTIVE DIRECTOR

The Arctic Institute of North America, The University of Calgary, is seeking applications for the position of Executive Director. The successful applicant is expected to hold a Doctorate degree from a recognized university, or equivalent qualifications; to have an established background in Arctic and Northern studies; university teaching experience, or an interest in teaching; and managerial ability. The Executive Director is a senior academic appointment liaising with the university administration through the Office of the Vice-President (Academic). Salary and benefits will depend on qualifications and experience.

Further details may be obtained from

Dr. K. E. Cooper
Associate Vice-President (Academic)
The University of Calgary
Calgary, Alberta T2N 1N4
Canada

to whom the letters of application together with a detailed curriculum vitae should be sent.

W359(A)

GUY'S HOSPITAL MEDICAL SCHOOL

GRADUATE PHYSICIST

required as Research Assistant participating in a radiology research project supported by the D.H.S.S., for the further development of panoramic (oral) techniques, with special reference to dosimetry and the application of xeroradiography.

This appointment provides an excellent training which would be valuable to a scientist wishing to enter an important growth area of radiology.

The appointment is until 30th April 1982 and the salary scale is £3,802 to £5,488 plus £740 London Allowance and superannuation.

Apply in writing, with full curriculum vitae and the names of two referees, to the Secretary, Guy's Hospital Medical School, London Bridge SE1 9RT, quoting Ref OM2. 3243(A)

UNIVERSITY OF CALIFORNIA
Berkeley

DEPARTMENT OF PHYSICS

RECENT PhDs

wanted for two positions in rare gas mass spectrometry opening between January 1 and April 1, 1980. Salary range $16,308 to $19,700 for one to two years. Experimental background required in research on planetary materials or geochemistry.

Appointees will have much independence in research on trapped rare gases in meteorites, rare gas dating of meteorites, and/or terrestrial rare gases.

Send by October 15, 1979, resume, statement of interests, and 2 or 3 references to Prof. J.H. Reynolds, Dept. of Physics, University of California, Berkeley, CA 94720. The University of California is an Equal Opportunity/Affirmative Action Employer. W372(A)

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF ORTHOPAEDIC AND ACCIDENT SURGERY

TECHNICIAN

for research project concerning the biological approach to replacement of arthritic joints. Facilities are available for cartilage and bone cell culture. Previous experience of immunological techniques desirable, but training given.

Candidates must possess HNC or appropriate equivalent qualification and have had several years laboratory experience.

Salary within a range £3,700 to £4,320 p.a., according to qualifications and experience.

Application forms available from the Registrar, The University, P.O. Box 147, Liverpool L69 3BX. Quote Ref: RV/777/N. 3215(A)

UNIVERSITY OF MANCHESTER

RESEARCH FELLOW IN RHEUMATOLOGY

Applications invited for this three-year appointment to work in the field of immunology, biochemistry and tissue culture work. The project involves basic research into the pathogenesis of rheumatoid arthritis and similar diseases. The work would be suitable for presentation as a PhD thesis, if not already held. The laboratory facilities and support are excellent.

The appointment would be available from September 15th, 1979. Salary range p.a. up to £5,400 (under review) according to qualifications. Further information from Dr P.J.L. Holt, University of Manchester Medical School, Department of Rheumatology, Manchester M13 9PT (Tel. 061-273 8241, Ext. 102). 3229(A)

FELLOWSHIPS

UNIVERSITY COLLEGE CORK
IRELAND

CORK HARBOUR MARINE BIOLOGY RESEARCH PROJECT
(N.B.S.T.)
2 Posts

A. POST-DOCTORAL RESEARCH FELLOWSHIP

A Zoologist is required for studies on the benthic ecology of Cork Harbour. The appointment will be for one year.

Applicants should have a PhD degree or equivalent experience.

Salary Scale: £4,182 to £4,845.

B. RESEARCH ASSISTANT

A Biologist is required to assist with Botanical and Zoological studies of Cork Harbour. The appointment will be for one year.

Applicants should have an honours degree in either Botany or Zoology or both.

Salary Scale: £2,919 to £3,941 per annum depending on qualifications.

Diving experience would be an advantage for either of the above positions but is not essential.

Applications for the above posts with curriculum vitae together with the names and addresses of 2 referees should be sent by **1st October, 1979** to Project Director, Dr. John Cullinane, Botany Department, University College, Cork. 3236(E)

RESEARCH FELLOWSHIP

A post-doctoral cytogeneticist or molecular biologist, with experience in human karyotyping, is required to participate in a project on the analysis of chromosomes from human solid tumours.

The appointment is for three years. Salary with entry according to qualifications and experience within range £5,990 to £7,434 inc. L.A. For further information telephone Dr E. Solomon 242 0200 extension 426.

Applications with curriculum vitae and names of two referees should be sent to the Secretary, Imperial Cancer Research Fund, Lincoln's Inn Fields, London, W.C.2., quoting reference 407/79. 3273(E)

THE HARTLEY FELLOWSHIPS of the UNIVERSITY OF SOUTHAMPTON

Applications are invited for three Fellowships normally tenable from 1 October 1980, for up to two years research, in any field for which facilities are available within the Faculties of Arts, Science, Engineering & Applied Science, Social Sciences, Law, Education, Medicine and Mathematical Studies.

Fellows will normally be appointed from persons of high promise whose records show evidence of capacity for original research, and who have up to five years post-doctoral experience or equivalent research record.

Salary will be related to age and experience and be within nationally agreed ranges for research staff in universities. (No provision for travel or removal expenses).

The closing date for applications for the 1980 competition is 1 November 1979.

Details and application forms from Mr E.D. Gordon, The University, Southampton SO9 5NH.

Please quote reference N. 3216(E)

POSTDOCTORAL FELLOWSHI
Transition state analogues, water effe on biological reactions. Experience synthetic or bio-organic chemis essential. R.V.Wolfenden, Department Biochemistry, University of No Carolina, Chapel Hill, N.C. 27514. UN an equal opportunity/affirmative act employer. W361(E

KING'S COLLEGE
Cambridge

JUNIOR FELLOWSHIPS in the Earth Sciences and the Physical Sciences

The College hopes to elect to Fellowsh to commence on 1 October 1980, in Earth Sciences (including Meteorology a Oceanography) and in the Physi Sciences (including Applied Mathemati

Men and women graduates are elig provided that they have not comple more than about three years of p graduate research by October 1979.

Further details from The Provost, Ki College, Cambridge CB2 1ST, to wh applications should be sent not later t 31 October 1979. 3272(E

THE HEBREW UNIVERSIT OF JERUSALEM

The Hebrew University of Jerusalem of a small number of

POST-DOCTORAL FELLOWSHIPS

for the 1980-81 academic year in Humanities, Social Sciences, Natu Sciences, Agriculture and Medicine.

Candidates may apply at an early stag their professional career (not later t three years after completion of t doctoral dissertation) to PO Box 12 Jerusalem, Israel.

Completed application forms must returned by December, 31, 1979.

3262(E

UNIVERSITY OF HAMBURG

Germany

POSTDOCTORAL FELLOWSHIP IN RECOMBINANT DNA RESEARCH

To study replication and expression of chemically synthesized DNA in bacteria. The applicants must have experience in the construction of plasmid or/and phage vectors and cloning of DNA in bacteria. It is of advantage to have performed experiments on the expression of recombinant DNA in bacteria. The position is at least for two years.

Salary of about DM 45000 p.a. Applications together with curriculum vitae, list of publications and the names of two referees should be sent to Prof. Dr H. Köster, Institut für Organische Chemie und Biochemie, Universität Hamburg, Martin-Luther-King-Platz 6, D-2000 Hamburg 13, FRG. W376(E)

UNIVERSITY OF NOTTINGHAM MEDICAL SCHOOL

DEPARTMENT OF BIOCHEMISTRY

POST-DOCTORAL RESEARCH FELLOW

Applications are invited for a research fellowship financed by an industrial company for a study of dietary phospholipids and liver lipid metabolism in relation to heart disease. The fellowship is tenable initially for one year from 1st October, 1979 or a date to be agreed. Renewal for a further two or three years is possible. Initial salary £4,333 with membership of U.S.S.

Applications with curriculum vitae and the names of two referees should be sent as soon as possible to Professor J.N. Hawthorne, Department of Biochemistry, University of Nottingham Medical School, Queen's Medical Centre, Nottingham NG7 2UH from whom further details may be obtained (telephone 0602-700111, Ext. 488). 3235(E)

IMPERIAL CANCER RESEARCH FUND

RESEARCH FELLOWSHIP

A post doctoral fellow is sought to join a group working on the structure and function of paporavirus tumour antigens, with particular emphasis on the possible role of membrane associated protein kinases in virus induced transformation. Experience in cell biology, membrane biochemistry, protein kinases or enzyme purification might be advantageous.

Three year appointment. Salary range 5,800 to £7,100. Further information from Dr Alan E. Smith (01 242 0200 ext. 39). Applications with curriculum vitae and the names of two referees should be sent to the Personnel Officer, Imperial Cancer Research Fund, Lincoln's Inn Fields, W.C.2. quoting reference 394/79. 3225(E)

Please mention

Nature

when replying to
these advertisements

FRIEDRICH MIESCHER-INSTITUT

POSTDOCTORAL FELLOWSHIP

IN BIOCHEMISTRY

is available for the area of ß-adrenergic receptor-mediated effects in tissue culture cells. The fellowship will run for two to three years.

Candidates should send c.v. and three names of referees to Dr M. Staehelin, Friedrich Miescher-Institut, PO Box 273, CH-4002 Basel, Switzerland. W364(E)

ST. GEORGE'S HOSPITAL MEDICAL SCHOOL

London SW17 0RE

POST-DOCTORAL RESEARCH FELLOWSHIP IN BIOCHEMISTRY/ IMMUNOLOGY

Applications are invited for the above post which is principally concerned with the biochemical and immunological characterisation of the cell surface of recently established human tumour cell lines. The appointment which is supported by the Cancer Research Campaign is for three years with the possibility of extension.

Applications and further particulars to Dr D A W Grant, Lecturer in Biochemistry, or Professor J Hermon-Taylor, St. George's Hospital Medical School, London SW17 0RE (01-672 1255, Ext. 4716). 3160(E)

UNIVERSITY COLLEGE DUBLIN

DEPARTMENT OF CHEMISTRY

Applications are invited for a Postdoctoral Fellowship in Bio-Inorganic chemistry. The work will involve a joint project with the Department of Agricultural Chemistry relating biological activity to the structure of metal chelates.

The Fellowship will be supported by the National Board for Science and Technology and will be for two years, commencing on February 1st, 1980. Salary will commence at £4,000 per annum.

Applications, accompanied by a curriculum vitae and the names of two referees, should be sent to: Professor D.A. Brown, Department of Chemistry, University College, Belfield, Dublin 4, Ireland. 3251(E)

UNIVERSITY OF BRADFORD

RESEARCH FELLOW

In Visual Science

Applications are invited for a 2 year Post-Doctoral Fellowship for research on the Characteristics of Normal and Defective Colour Vision. The successful candidate will be attached to the new Chair in Visual Science and will work under the direction of Professor J D Moreland. It is hoped to make the appointment as soon as possible. Salary within range £4,333 to £7,521 pa (under review). Superannuable.

Further particulars and application forms (to be returned asap) from the Registrar, Post Ref VS/RF/R/X, University of Bradford BD7 1DP, West Yorkshire. 3252(E)

THE LADY DAVIS FELLOWSHIP TRUST
PO BOX 1255, JERUSALEM, ISRAEL

FELLOWSHIPS FOR STUDY AND/OR RESEARCH AT GRADUATE OR POST-DOCTORAL LEVELS AND VISITING PROFESSORSHIPS

in 1980 — 81

at the Hebrew University of Jerusalem and at the Technion — Israel Institute of Technology, Haifa

GRADUATE AND POST-DOCTORAL FELLOWSHIPS

Eligibility: Lady David Fellows will be selected on the basis of demonstrated excellence in their studies, promise of distinction in their chosen fields of specialization and qualities of mind, intellect and character.

Post-doctoral candidates shall have completed their doctoral dissertation before arrival in Israel.

The Graduate and Post-doctoral Fellowships are tenable for a period of one year. They may be renewed for a second year and in special circumstances extended to a third year.

The grant is intended to defray the cost of the Fellow's travel and tuition fees and to meet reasonable living expenses.

Application: Graduate candidates may apply during their senior academic year or after they have undertaken study in a graduate school. Post-doctoral candidates may apply at an early stage of their professional career (not later than 3 years after completion of their doctoral dissertation).

Graduate and Post-doctoral candidates (including Israelis abroad) may obtain application forms from the Lady Davis Fellowship Trust at the above address, after they indicate the category of Fellowship for which they are eligible.

Completed applications must reach the office of the Trust not later than January 1, 1980.

VISITING PROFESSORSHIPS

These Fellowships are intended for candidates with the rank of Full or Associate Professor at their own institution. They are tenable for periods from one trimester (or semester) to a year.

Application: Forms may be obtained from the Lady Davis Fellowship Trust at the above address. Completed application forms for the Hebrew University must reach the office of the Trust by November 30, 1979. Those for the Technion — by December 31, 1979. 3263(E)

SEMINARS and SYMPOSIA

EORTC SYMPOSIUM

Immunopharmacologic Effects of Radiotherapy

Montpellier, France
13th 14th and 15th March 1980

The program will include invited and proferred papers.

The meeting is designed to promote discussions among following topics:

Effects of ionizing radiation on the immune response: clinical and experimental aspects.

Immediate, secondary and long term incidences of the radiotherapy on the immune response and on the host-tumor relationship in cancer patients.

— Correction or modulation of the changes induced by radiotherapy.

— Therapeutical associations: radiotherapy — immunotherapy; radiotherapy — chemotherapy; immunotherapy: experimental protocols, clinical trials and on-going studies.

Free papers will be submitted to the Scientific Committee.

The proceedings and reports from invited speakers will be published.

Abstracts, subscription and registration fees (600 FF or 140 Dollars) are to be sent not later than February 15th 1980 to the General Secretary.

The number of inscriptions will be limited to one hundred.

Organizing Committee:
Dr J.B. Dubois (Montpellier)
Dr B. Serrou (Montpellier)
Dr Cl. Rosenfeld (Villefjuif)

Further information can be obtained form Dr J.B. Dubois, General Secretary, Centre Paul Lamarque, Hopital Saint Eloi, B.P. 5054, Montpellier, France. W358M

ASSISTANTSHIPS

UNIVERSITY OF NOTTINGHAM

DEPARTMENT OF APPLIED BIOCHEMISTRY AND NUTRITION

(Food Science)

RESEARCH ASSISTANTSHIP

for a Study of Interactions between Proteins and Polysaccharides in Food Systems

Applications are invited for a

RESEARCH ASSISTANT

to work with Drs D.A. Ledward and J.R. Mitchell on the interactions between proteins and polysaccharides in some novel food systems. The project is supported by a grant from the Agricultural Research Council for a maximum period of 3 years. The salary will be in the range £3,775 to £4,333 p.a. (under review) and the successful applicant will be expected to register for a higher degree. The project will involve both fundamental and applied studies and honours graduates in chemistry, food science, biophysics, biochemistry or related discipline are invited to apply.

Applications with curriculum vitae including the names of two referees should reach the Secretary, University of Nottingham, School of Agriculture, Sutton Bonington, Loughborough, Leics. LE12 5RD, as soon as possible. 3217(P)

Public Health Laboratory Service

Centre for Applied Microbiology & Research, Porton Down

Diagnostic Reagents Laboratory

Post-Graduate Research Assistantships

Two posts are available to join a small research team working on genetics and genetic manipulation in bacilli. Applications are invited from well qualified graduates with a degree in Biochemistry, Microbiology, or related subjects, for:

1. Research on the development of genetic manipulation in bacilli and other gram-positive microorganisms. Applicants should have a good background knowledge of Microbial Genetics/Molecular Biology and be interested in the existing techniques of Genetic Manipulation.
2. Research into the genetics of thermophilic bacilli and their bacteriophage. Applicants should have a strong interest in Microbial Genetics/Molecular Biology.

The posts are funded by a research contract with the Health & Safety Executive and are tenable for up to 3 years.

Salary will be in the range £3,486 to £4,935 increasing to £3,807 to £5,316 on 1.1.1980. NHS terms and conditions of service.

These posts may be discussed informally by contacting A. Bingham or R. Sharp; Tel. 0980-610391, Ext, 371/394.

PHLS

Applications including curriculum vitae and two academic referees should be sent to Mrs. M. Bushby, Personnel Officer, CAMR, Porton Down, Salisbury, Wilts. Closing date 5 October 1979.

3246(P)

THE UNIVERSITY OF SHEFFIELD

DEPARTMENT OF MICROBIOLOGY

Applications are invited for a Postdoctoral Research Assistantship financed by the SRC for work on biochemical and genetic aspects of anaerobic energy metabolism in *E.coli* involving DNA cloning and the control of expression of several cloned functions. Tenable from October 1979 for three years. Starting salary up to £5,199 a year.

Applicants should possess or expect soon to possess a PhD in molecular biology, genetics, microbiology or biochemistry.

Applications, including curriculum vitae and names of two referees to Dr JR Guest, Department of Microbiology, the University, Sheffield S10 2TN as soon as possible. Quote ref: R364/G. 3248(P)

THAMES POLYTECHNIC

SCHOOL OF BIOLOGICAL SCIENCES

RESEARCH ASSISTANTSHIP

Applications are invited for a Research Assistant to undertake, under the supervision of Dr A R W Smith, an investigation into the pathway of degradation of the herbicide 2,4-dichlorophenoxy acetic acid (2,4-D) and its induction in *Acinetobacter* sp. The post is tenable from 1 November 1979. Applicants should hold a good honours' degree in biochemistry or chemistry, and some experience in microbiology is preferable, but not essential.

Salary scale: £3,321 to £3,705 inclusive.

Further particulars and form of application may be obtained from the Staffing Officer, Thames Polytechnic, Wellington Street, London SE18 6PF, to whom completed applications should be returned by 2 October, 1979. 3245(P)

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New York
Suite 832
50 Rockefeller Plaza
New York, NY 10020
(212) 765-5758

THE UNIVERSITY OF SHEFFIELD

DEPARTMENT OF CHEMISTRY

Applications are invited from men and women for an

SRC POSTDOCTORAL RESEARCH ASSISTANTSHIP

to study the photochemistry of carbanions and, in particular the cis-trans photoisomerisation of diphenyl-allyl and related anions. Experience of vacuum techniques for handling reactive compounds and/or photochemistry or photophysics (flash photolysis, measurement of fluorescence lifetimes and spectra) needed. Tenable for up to three years from 1 October 1979 or as soon as possible thereafter. Salary on range 1A at a point dependent on age and experience. Applications naming two referees to Drs B. Brocklehurst and R.N. Young, Chemistry Department, The University, Sheffield S3 7HF, from whom further information can be obtained. Quote ref: R343/BF/G 3256(P)

UNIVERSITY OF DURHAM

DEPARTMENT OF PHYSICS

Applications are invited from graduates, preferably with PhD in the field of High Energy Astrophysics, for a

POSTDOCTORAL SENIOR RESEARCH ASSISTANTSHIP

tenable for three years from as soon as possible. The successful candidate will join a research group working in experimental Gamma Ray Astronomy and should preferably have some experience in the field of digital and analogue electronics. Experience with balloon borne experiments would be an advantage.

Salary on National Range 1A £4,333 to £7,521 per annum plus superannuation.

Applications (3 copies) naming three referees should be sent by 28 September, 1979 to the Registrar and Secretary, Science Laboratories, South Road, Durham DH1 3LE, from whom further particulars may be obtained. 3271(P)

FOR SALE and WANTED

THE MYTH OF SCIENCE, or The fantasy of truth by Frederick R. Schram

Tells what science is and how it is done, what its limitations and weaknesses are and how these can affect public and private life. Focuses on 2 common myths: that science is methodical and objective, and scientists are rational, open-minded men and women. Examples from history and recent headlines make timely and hard hitting points.

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New York, N.Y. 10001

W366(L)

FOR SALE: OPTOMAX (Micromeasurements) Modular Image Analyser includin CPU, Power supply, Video Moniter Flexidraw Module, Circular Variabl Frame Module and Averaging Unit. Les than two years old; little used. Pleas contact: Ms Margaret Androu, Scott Pola Research Institute, Lensfield Road, Cambridge, CB2 1ER.

3158(L)

GRANTS and SCHOLARSHIPS

WELSH NATIONAL SCHOOL OF MEDICINE (University of Wales)

DEPARTMENT OF PHARMACOLOGY

RESEARCH SCHOLARSHIP

Applications are invited fro Pharmacologists or Biochemists holding 1st or 2(i) honours degree for a projec investigating neurotransmitter metabolis in Huntington's Chorea.

The Scholarship will be available for years and the successful candidate woul be expected to apply for registration for a MSc degree.

Applications, together with the name and addresses of two referees should b sent as soon as possible to Dr J.A. Davies Department of Pharmacology, Wels National School of Medicine, Heath Park Cardiff, CF4 4XN from whom furthe information is available. 3240(H)

ASSOCIATESHIPS

UNIVERSITY OF LEICESTER

DEPARTMENT OF PHYSICS

RESEARCH ASSOCIATESHIP IN SURFACE PHYSICS

Applications are invited for the post of post-doctoral research associate to work on a project concerned with the study of metal and alloy surfaces using angle-resolved photoelectron spectroscopy at the National Synchrotron Radiation Facility at Daresbury. Experience of photoelectron spectroscopy or surface techniques would be an advantage. The post is available for up to three years from October 1st 1979 and the salary is from £4,333 p.a. The work is supported by the Science Research Council.

Applications, including a curriculum vitae and names and addresses of two referees should be sent to Dr C. Norris, Department of Physics, University of Leicester, Leicester LE1 7RH. 3221(O)

National Research Council Canada   Conseil national de recherches Canada

1980
RESEARCH ASSOCIATESHIPS

for research in science and engineering in the following laboratories of the

NATIONAL RESEARCH COUNCIL OF CANADA

DIVISION OF BIOLOGICAL SCIENCES
DIVISION OF BUILDING RESEARCH
DIVISION OF CHEMISTRY
DIVISION OF ELECTRICAL ENGINEERING
HERZBERG INSTITUTE OF ASTROPHYSICS
INDUSTRIAL MATERIALS RESEARCH INSTITUTE
DIVISION OF MECHANICAL ENGINEERING
NATIONAL AERONAUTICAL ESTABLISHMENT
DIVISION OF PHYSICS
PRAIRIE REGIONAL LABORATORY

Applicants should possess at least a PhD in natural science or a Master degree in an engineering field or expect to obtain the degree before taking up the Associateship.
Associateships are open to nationals of all countries, although preference will be given to Canadians.
Research Associates will be offered appointments to the staff of the National Research Council on a term basis and will be offered salaries and benefits currently available to members of the continuing staff.
The initial appointment will be for a one-year term and may be renewed, subject to the Associate's performance and subject to the requirements of the division. Renewals are considered annually. The term as a Research Associate will vary by division, but will not exceed five years.
An allowance will be provided towards the cost of travel.
Applications may be obtained from the Research Associates Office, National Research Council of Canada, Ottawa, Canada, K1A 0R6.
Closing date: 15 January 1980 W375(O)

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- 8 JAN 195

Advanced techniques in routine use at The Radiochemical Centre

Described below are just two examples of the many up-to-date techniques, which have been pioneered or applied for routine use at The Radiochemical Centre. These developments are part of our constant endeavour to maintain our position at the forefront of the specialised field of tracer methodology, so that we can continue our supply of radiochemicals of the highest quality and technical specifications.

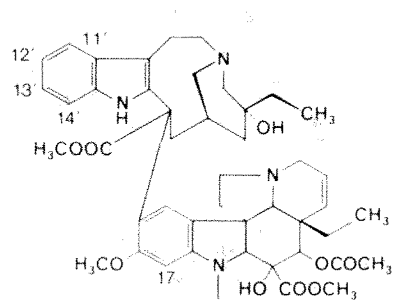
Distribution of labelling in tritium compounds

Modern techniques for the production of tritiated compounds are more sophisticated than those used in the early days of tritium labelling, and produce compounds labelled in specific positions rather than generally labelled. Nevertheless, it is necessary for many tracer applications of tritium compounds to know the *precise* position and configuration of the tritium labels. Traditional chemical methods of doing this are tedious and time consuming and subject to considerable error, and so the routine supply of such information has until recently not been possible.

The Radiochemical Centre, in collaboration with the University of Surrey, has developed over the past eight years the technique of tritium nuclear magnetic resonance (tnmr) spectroscopy for this purpose. This method is much quicker and more accurate than the traditional chemical or biochemical methods for determining distribution of tritium labelling.

It is now used routinely to establish the distribution of tritium labelling produced by the usual methods of tritiation employed at The Radiochemical Centre. We supply accurate details as to the position and configuration of the tritium labels for an increasing number of our labelled compounds.

A number of examples are given below:



[G-³H]VINBLASTINE
Code TRK.507

17	44%	11′	8%
12′,13′	46%	14′	2%

[1β, 2β(n)-³H]TESTOSTERONE
Code TRK.162

1α	5.9%	2α	8.2%
1β	46.0%	2β	39.9%

[6-³H]BENZO[a]PYRENE
Code TRK.501

6	>95%	1	<5%

D-[2-³H]GLUCOSE
Code TRK.361

2(α)	40%	2(β)	60%

L-[3,4(n)-³H]VALINE
Code TRA K.533

2	8%	4	64%
3	28%		

The latest of our publications is as follows:
AL-RAWI, J.M.A., BLOXSIDE, J.P., ELVIDGE, J.A., JONES, J.R., CHAMBERS, V.E.M., CHAMBERS, V.M.A. and EVANS, E.A., *Steroids* vol. 28 (3), p.p. 359-375, 1976.

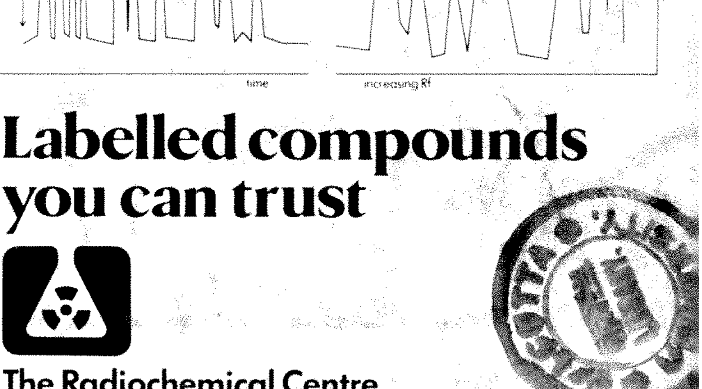
High performance liquid chromatography (HPLC)

This relatively new development of column chromatography is carried out using high efficiency microparticulate column packings of closely defined size.

Chromatography is carried out under pressure to ensure good flow rates and reduce diffusion of separated compounds. Dead volumes are kept to an absolute minimum. The result is that many separations can be carried out more quickly and with better resolution than with previously used chromatographic methods such as thin-layer chromatography or conventional column chromatography.

Work aimed at developing the applications of this method to radiolabelled compound separations is still in progress, but The Radiochemical Centre is already using the technique in many of its production processes, and in analytical applications. The result is purer compounds for the customer and greater efficiency of working.

The example illustrated below illustrates the clear superiority of HPLC when used as an analytical tool. The mixture used comprised the tritium labelled mono-, di- and triphosphates of adenosine, cytidine, guanosine and uridine, and all are clearly separated in the HPLC system.



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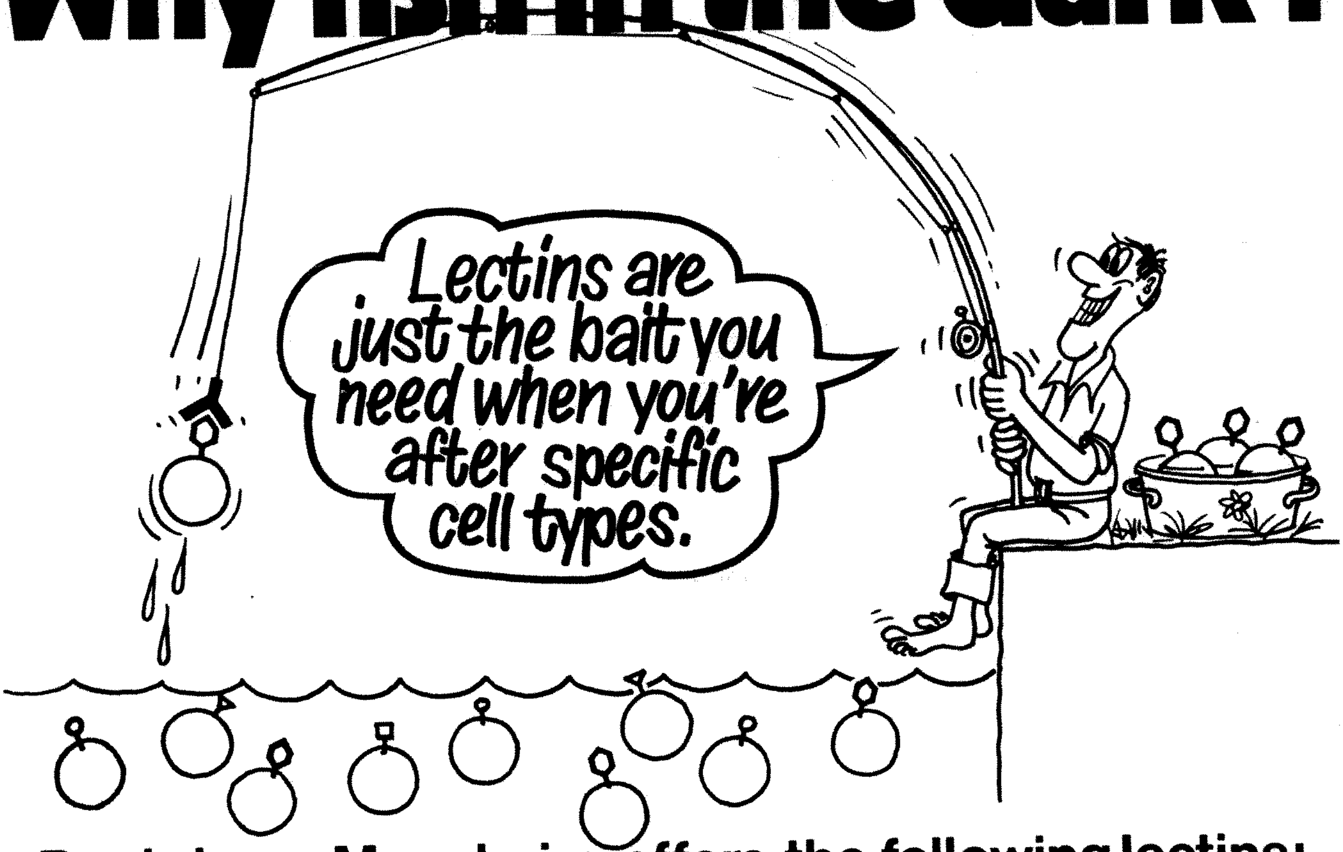
Lower Carboniferous Flora in Indi

Why fish in the dark?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Lectin	Source	Sugar Specificity	Cat. No.
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Ricin	Ricinus communis	β-D-Gal>α-D-Gal>D-GalNAc	223743
Lectin from R. communis (RCA 120)	Ricinus communis	β-D-Gal> α-D-Gal	223727
Lectin from peanuts (PNA)	Arachis hypogea	β-D-Gal-(1→3)-D-GalNAc> D-GalNH$_2$ = α-D-Gal	223697
Lectin from soybeans (SBA)	Glycine max	α-D-GalNAc> β-D-GalNAc>> α-D-Gal	373206
Lectin from H. pomatia (HPA)	Helix pomatia	α-D-GalNAc> α-D-GlcNAc>> α-D-Gal	373168
Lectin from wheat germs (WGA)	Triticum vulgaris	β-D-GlcNAc-(1→4)-β-D-GlcNAc-(1→4)-GlcNAc> β-D-GlcNAc-(1→4)-β-D-GlcNAc	166006
Concanavalin A (Con A)	Canavalia ensiformis	α-D-Man> α-D-Glc>α-D-GlcNAc	103594
Lectin from lentils (LA)	Lens culinaris	α-D-Man> α-D-Glc> α-D-GlcNAc	165964
Lectin from P. americana (PWM)	Phytolacca americana (Pokeweed mitogen)	Di-N-acetylchitobiose (Yokoyama, K. et al. (1978) Biochim. Biophys. Acta, **538,** 384)	373184

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Telephone: (01) 836 6633 Telex: 262024
Telegrams: Phusis London WC2R 3LF

Editor: David Davies
Deputy Editor: Peter Newmark

Editorial Staff
Philip Campbell Sara Nash
Isobel Collins Judy Redfearn
Patricia Dent Miranda Robertson
Konrad Guettler Robbie Vickers
Eleanor Lawrence Robert Walgate
Mary Lindley Charles Wenz
Jonathan Wolfe

Publishing Director: Chris Paterson

New York
175 Fifth Avenue, New York, NY 10010
Telephone: (212) 777 5858 Telex: 668497
Robert Ubell (American Publisher)
and Sheila Kane

Washington News Bureau
711 National Press Building, DC 20045
Telephone: (202) 737 2355 Telex: 64280
David Dickson (Washington News Editor)

London Advertisement Manager:
Richard Webb
3 Dyers Buildings, London, EC1N 2NR
Telephone: (01) 831 6901

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Fossil *Rhacopteris* from the Gund Formation, Kashmir. This new discovery, reported on page 295, extends the realm of the *Lopidodendropsis* flora of the Lower Carboniferous to other parts of India.

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Guide to authors

- Review articles should be aimed at a relatively wide readership. Many reviews are invited, but submitted articles may also be accepted; it is advisable to consult us before writing a review article.
- Articles may be up to 3,000 words long with at most six displayed items (figures and tables); they are reports of major research developments.
- Letters are brief reports of original research of unusual and wide interest, not in general longer than 1,000 words; they have at most three or four displayed items.
- 'Matters Arising' permits short discussion (up to 300 words) of papers that have recently appeared in *Nature*.

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Manuscripts may be submitted either to London or New York. Three typed copies should be submitted, each including lettered copies of figures. Typing (including references) should be double spaced. The title should be brief and informative. Pages should be numbered. References, tables and figure legends should start on separate pages. Experimental detail vital to the paper yet which would interrupt the narrative is best placed in the figure legends. Units should conform to the Système International. Greek characters should be identified in the margin on their first appearance. Equations should occupy single lines if possible; exp (a) is preferred to e^a if 'a' is more than one character.

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David Davies, the present Editor of *Nature*, will be leaving at the end of 1979 to become Director of the newly-formed Dartington North Devon Trust. The purpose of the Trust will be to find ways of improving life materially and otherwise in a rural area, through industrial innovation and through the introduction of new ideas in education, housing, leisure and the arts.

An announcement concerning the appointment of a new Editor is on page iii, opposite.

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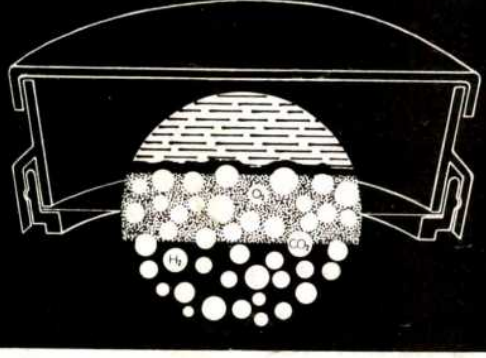

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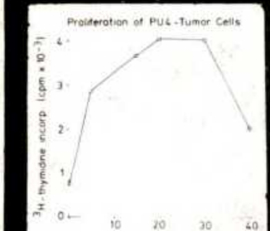

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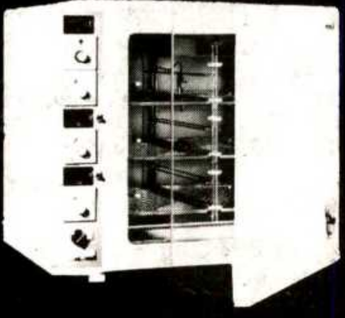

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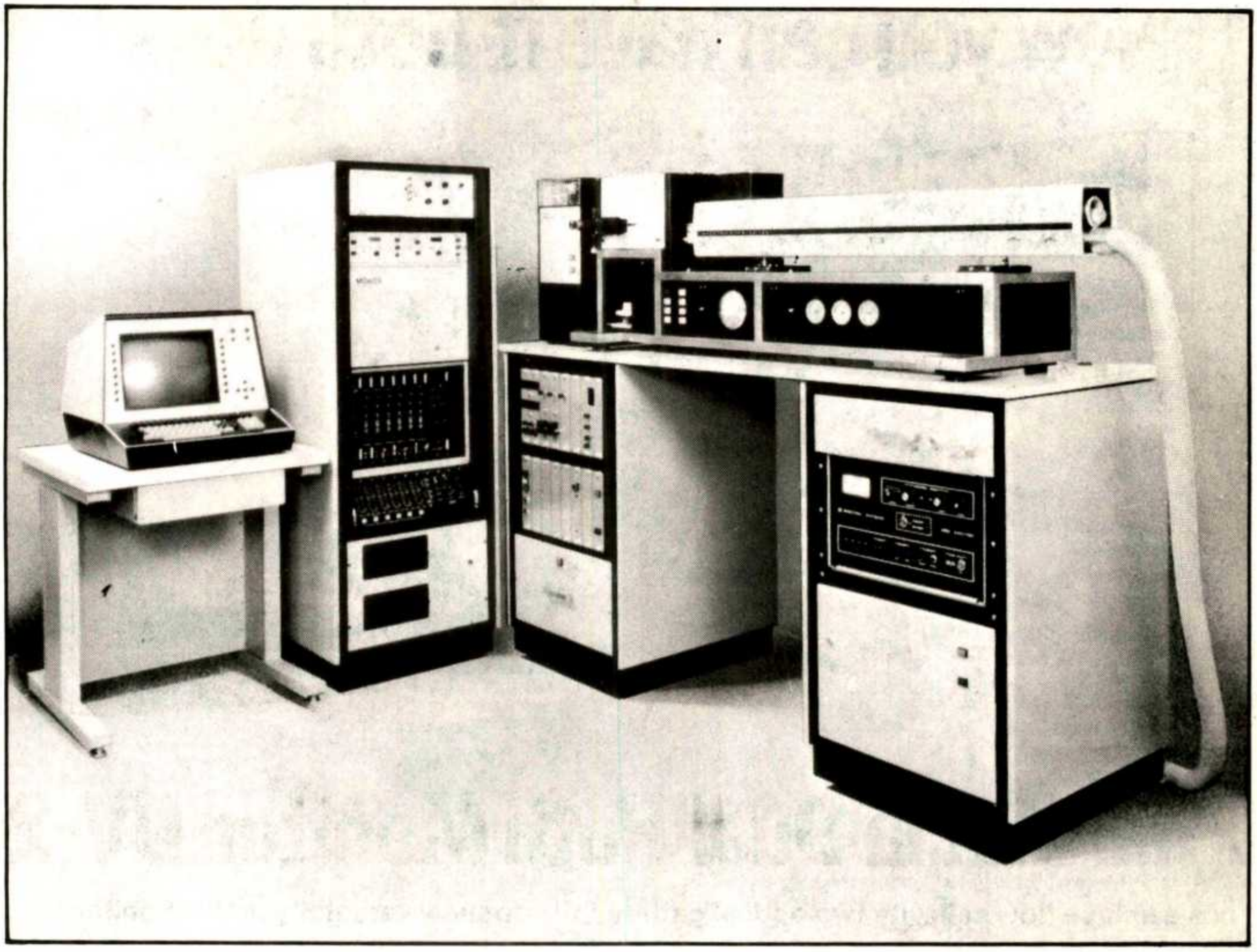

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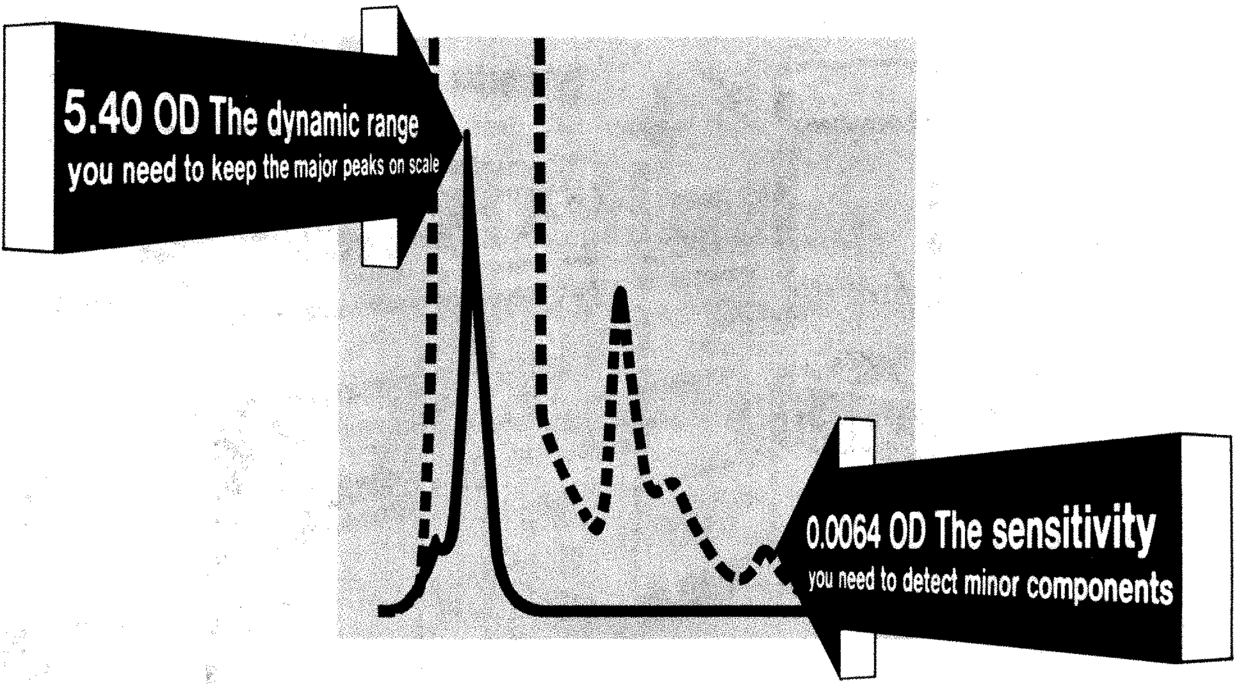
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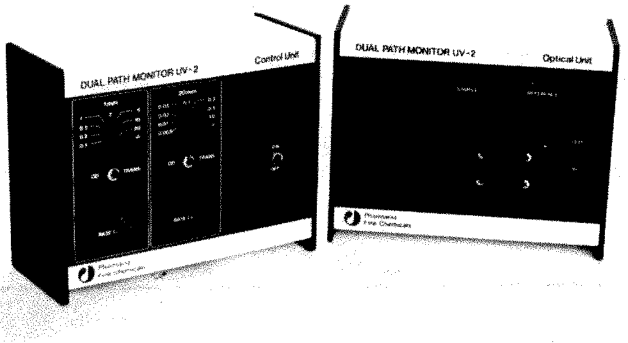

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The nuclear 1980s

Two events coming up in 1980 will influence the world's nuclear future for many years. In the spring the International Nuclear Cycle Fuel Cycle Evaluation (INFCE) will issue a report that is the result of nearly two years of study by eight working groups. Then a few months later, upwards of one hundred nations — signatories to the Non-Proliferation Treaty (NPT) — will convene for their second five-yearly review of the treaty. The events are by no means unrelated. They both reflect efforts in the world community to come to grips with the problem of capitalising on the peaceful benefits of nuclear power without opening the door to nuclear weapons. And neither, in their different ways, will be able to hold out much hope that there is any simple way forward, either in terms of technological fixes or new institutions, which will make the 1980's a time when nuclear weapons stop spreading into yet more hands.

INFCE, the backwash to President Carter's 1977 initiatives to arrest proliferation by unilateral and widely disliked measures, is likely to deliver a fairly comfortable message to nuclear states — not great surprise given the representation of nuclear enthusiasts on the working parties and the absence of any brief to consider broader issues such as environment or safety. At a meeting of the British International Studies Association last week, Dr S. Warnecke indicated that the conclusions will probably run something like this: there is no geological shortage of natural uranium although there could be political problems in international sales; if there is need for some more enrichment capacity this should be confined to technologically advanced countries (such as Japan); there is wide divergence between countries on the economic sense of reprocessing (the United States failed to get any measure of consensus on the desirability of once-through cycles); the non-proliferation risks associated with fast-breeders are no greater than those of light-water reactors; and there is as yet nothing in advanced reactor concepts which would inherently make them more proliferation-proof than current designs. In short, the path that major nuclear nations have been taking these past ten years is neither free of proliferation risks nor is it recklessly dangerous, and no other path holds out any obvious attractions of more proliferation-proof power. This is hardly going to be a surprise to the nuclear bureaucracies of the world, although it may well disappoint President Carter who at one time hoped that INFCE would provide some international agreement on positive steps that could to be taken to keep the danger of proliferation out of the nuclear fuel cycle.

The message of INFCE to the NPT Review Conference will be, then, that it is difficult if not impossible for the international technoligical community to do much in the final analysis to prevent the spread of nuclear weapons — a nation with a strong political will to go nuclear can easily overcome such technical obstacles as are put up. This gloomy view will coincide with unencouraging news on other fronts. On Article VI of the treaty, in which nuclear-weapon states promise as their part of the bargain to work for disarmament, there is relatively little to present except an uncertain and unimpressive SALT 2, and glacial advances towards an almost meaningless (because temporary) comprehensive test ban treaty. Futhermore the last five years have seen the emergence of a different and ominous sort of proliferator — the nation which, because of heavy hints, must be assumed to be acquiring a nuclear weapons capability even though it doesnt take the final step of firing a device.

Can the NPT survive all this? In a formal sense, yes; no doubt for the next few years it can retain all its signatories, and it must never be forgotten that there are some very desirable names on the list. But as time progresses the treaty looks more and more like every other arms control measure — a limitation only on those who have no particular desire to transgress. □

. . . and the broadcasting 1990s

The World Administrative Radio Conference starts this week in Geneva and is expected to run until December. Public interest is likely to focus on the allocation of frequencies, and more particularly the North-South and East-West political conflicts inherent in these allocations. But one issue which could do with much more public exposure is the question of the accessibility of television transmissions.

Radio broadcasts, with the help of the ionosphere, know no national frontiers, and as a result there is a richness of choice open to anyone with even a modest receiver. The present, ensured that it has been much more of a national affair. As a consequence access to a mass audience has been in the hands of a relatively small number of people, all with similar cultural backgrounds.

Satellite television could change all this. Within ten or fifteen years it could be possible to tune in to French, German, Italian or Russian television by directing a rooftop aerial at the appropriate satellite. The trouble is that not every country wants its people to have that freedom, which the objectors term cultural or political subversion.

As a result a 1977 conference went to great lengths to ensurey, by means of satellite placings, polarisations and radiation patterns, that the cause of international understanding is not going to be given much of a boost by television.

Time is running out for brave enterprises to resist this narrow nationalism. The Geneva meeting could be the last opportunity for anyone to raise a voice against it. □

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Battle heats up over control of workers' health

The US Occupational Safety and Health Administration will soon face one of the severest political challenges of its ten-year existence. In the second of three articles on the current anti-regulatory mood in Washington **David Dickson** reports on pressures on the agency and their implications for environmental research

Within the next few weeks a Senate Committee will begin discussing two bills either of which could, if implemented by Congress, significantly restrict the activities of the Occupational Safety and Health Administration (OSHA).One proposes a partial exemption for a large number of companies from the provisions of the act which set up the agency in 1970; the second is a stronger bill which would mean total exemption for small businesses removing the agency's jurisdication over more than four million workers.

Attempts to limit the activities of OSHA — perhaps the federal agency least loved by the US business community — have frequently been launched in Congress, but have seldom gathered much momentum. This year, however, the situation of different. Defenders of the agency have less support than previously; while critics, buoyed by their success in securing an amendment to OSHA's appropriations bill which effectively bars the agency from routine safety checks on 1.5 million establishments, are confident that at least one of the new bills may reach the statue book.

Disenchantment with OSHA has been directly fuelled by the anti-regulatory mood that is increasingly dominating both the Congress and the administration. Much of this has its roots in the economic argument that excessive regulation has been an important contributor to inflation, in particular reducing the rate of technological innovation and thus slowing productivity growth.

But behind this there is a political argument over who should have prime responsibility for protecting the health of the worker. Whether it should be the federal government, in its role of social regulator; or whether the responsibility should be passed partially back to private industry, operating under broad government guidelines, but responding primarily to economic incentives and the dictates of the market-place.

Support for the latter view is growing in strength. But to the extent that such sentiments have already been relected in various Congressional actions, they have been strongly condemned by the trade unions. In a report on recent and current legislative moves, for example, the United Steelworkers of America says that 1978 and 1979 were "dismal" years in Congress for workers safety and health.

"The errant pot shots that had been fired at OSHA every year since its enactment have now improved in their accuracy and their potency, and real erosion is beginning to take place", the union says.

The seeds of the present conflict between supporters and critics of OSHA were sown in the 1970 act establishing the agency. This requires OSHA to work towards achieving a "safe and healthful" environment for every US worker — but gave little specific indication as to which criteria should be used to make this assessment.

Initially OSHA came under steady fire for appearing to concentrate on "nit-picking" issues, such as the height of fire extinguishers above the floor, and ignoring many of the more important health and safety standards.

Its image of ineffectiveness and petty-minedness has been successfully revamped under OSHA's present head, Dr Eula Bingham. Appointment by President Carter in 1977, Dr Bingham has shifted the main emphasis from safety to health — a move reflected in the fact that OSHA has produced more health standards in the last two years than it did in the previous six.

Agency officials point to one of its major successes as being an increased public awareness of the importance of occupational health as a social issue. But they acknowledge the paradox that this has occurred precisely at a time when there are growing demands for the government to reduce its regulatory activities; and that this puts OSHA and its supporters in a vulnerable situation.

Evidence of this shift in public mood is provided by the speed with which even "liberal" politicians are now jumping on the regulatory band-wagon. The more restrictive of the two proposed Senate bills, for example, has been submitted by Senator Franch Church of Idaho, considered a liberal in US politics, but facing a tough challenge from the right in his re-election battle next year.

"Having someone like Church, who we do not consider an enemy, against you makes things a lot harder to deal with," says George Taylor of the AFL-CIO (American Federation of Labour — Council of Industrial Organisations), who foresees a need for the unions to "fight like hell" to protect some of the gains made in recent years.

It has become politically acceptable to challenge the activities of regulatory agencies. Speaking at a meeting of the American Chemical Society in Washington two weeks ago, for example, Dr P M Nortling, director of health and safety for the Du Pont Chemical company, said that although the number of citations for illegal practices issued by OSHA had diminished over the past two years, the proportion challenged by industry had almost doubled, from seven to 12% over this period.

Corporate attitudes

"There has been an important change in corporate attitudes. In the past, corporations were unwilling to appear obstructive on health and safety issues. That reluctance is now giving way, and we are now seeing the chemical industry taking a very active role in challenging regulatory proposals, particularly those which we feel impose economic burdens incommensurate with the expected benefits," Dr Norling said.

Ironically many of the current controversies result from a surfeit of laboratory data about the physiological effects of particular substances — at least when compared to the relative lack of epidemiological data on human health implications of the type which can be used as a solid basis for regulation.

A typical case in point it OSHA's proposal to implement "generic" carcinogen controls and standards. Under this proposal, first put forward two years ago and expected to be published in its final form next month, the agency will classify as a carcinogen any substance known to produce tumours in any single species of laboratory animal — and will demand that

Above: working in a steel foundry

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occupational exposure to this substance should be reduced as low as technologically feasible.

OSHA scientists argue that, given the many uncertainties that surround the mechanisms of carcinogenisis, workers should be given the benefit of the doubt. And this means protecting them against any substance bearing the least suspicion of carcinogenic properties.

The chemical industry, on the other hand, has attacked this approach as being both unnecessarily expensive — it quotes a cost for implementing the proposals as between $8 and $88 million — and as inherently "unscientific". The industry argues that more substantial data is required before taking such drastic action; this might, for example, include classfying carcinogens in terms of relative potency.

The industry has formed a lobbying group, the American Industrial Health Council, specifically to challenge OSHA's proposals — referred to last week as an "administrative short-cut" by AIHC executive director Ronald A Lang.

One AIHC proposal meeting with more sympathy in the administration, however, is that there should be a clearer distinction between the scientific and the political decisions made in the regulatory process. The AIHC has proposed that the National Academy of Sciences appoint a panel of "the best scientists the government can tap" to assess whether a particular substance is or is not a potential human hazard, and if so to what degree. The appropriate control mechanisms would them be agreed by a separate body.

A similar, although not identical, two-stage process for dealing with occupational hazards is already being developed within the administration, using the Department of Health, Education and Welfare's National Toxicology Programme to centralise the scientific assessment of potential hazards; and further developments along these lines are expected to be contained in a report from the President's newly apointed Regulatory Council, due in a few weeks time.

Even though such centralisation may have advantages in terms of efficiency, however, not everyone is totally convinced of its merits, some fearing that it might put a strait-jacket on the flexibility made possible by a plurality of programmes. "If there is an indication that these joint programmes are used to explore certain questions and to neglect others, that will be a real problem," says Dr Robbins of the National Institute of Occupational Safety and Health.

But the pressures to change the whole shape of Washington's regulatory apparatus continue to build, Until a few months ago, the dominant demand was that this apparatus should be "rationalised" in the interests of cost effectiveness. More recently, there has been what Dr Frank Press, director of the Office of Science and Technology Policy, has referred to as the emergence of a "new philosophy" emphasising economic incentives rather than regulatory clout.

Speaking at the ACS meeting two weeks ago, Dr Press suggested that industry should be given more flexibility in deciding how to achieve overall regulatory goals established by society. This could be done, for example, by applying the "bubble concept" used for air pollution control — requiring merely that a plant's total effluence release complies with federal standards — to health and safety.

"One of the important things to recognise regarding this approach to regulation is that it provides new incentive to industry to innovate. It takes some of the repressiveness and uncertainty out of regulation, and relies more on market-forces to achieve the desired goals in environmental quality, health and safety."

New legislation along these lines may well emerge in the next few weeks, when President Carter announces the conclusions that he has reached following a year-long review by the Department of Commerce of the effects of government policy on industrial innovation; the forty recommendations passed to the White House for presidential decisions include a number on regulatory reform, including for example a proposal to shift from design to performance criteria as the basis for regulation.

A number of observers in Washington feel that, with an election year approaching, it would be difficult for the President to support any major roll-back in occupational safety legislation — if only because trade union support is probably more important than that of environmentalists who have complained of the impact of proposed energy legislation.

But within the administration there is considerable dispute over whether the incentives approach is the right one to take. OSHA, for example, has been strongly opposed to demands that health and safety regulation should be justified on economic grounds, arguing in particular that there is no way that a worker's health can be adequately quantified — and certainly not by putting a market value on it.

US textile worker: stringent limits to cotton dust exposure have been one of OSHA's recent successes

Indeed almost all sides in the dispute on an appropriate policy now seem to accept that the vagaries of cost-benefit analysis are such as to make it an inadequate basis for regulatory decision-making — and that no statistic can really be trusted to give a full reflection of either the costs of regulation or its benefits.

As far as Congress is concerned, the response has been explicitly political. "Ultimately out belief is that the protection of the work-force is the responsibility of the employer, working with the employee, not of the OSHA policeman holding a club" says Mark de Bernardo of the Chamber of Commerce, a Washington-based group representing private industry interests which is providing active support to legislative attempts to change OSHA's mandate.

Trade unions

Trade unions and their supporters, however, see things differently. Although critical of the relative lack of impact which OSHA has had in the past, they credit it with many important gains, for example some of its recent programmes in worker education. And they are convinced that the health of workers can be better achieved through an effective regulatory agency rather than through the collective bargaining process.

"Protecting the gains made by OSHA is going to require vigilance and hard work," says George Coling of the Urban Environment Congerence, a lobby group which has been engaged in developing a coalition of labour, environmentalist and minority groups on occupational health issues. "It is not a battle for the agency, but it is a battle for health — and the agency is the tool that we need."

Ironically, the one aspect of federal health and safety efforts which has so far been relatively immune from Congressional attack has been the research budget. The budget for NIOSH, for example, is scheduled to increase by 25 per cent for the fiscal year 1980, reflecting the administration's commitment, articulated through the science adviser's office, to improve the "science base" of its various spheres of activity.

Yet even this may have its price. "It is possible that Congress may want to put more money into research rather than into adequate reinforcement. We obviously welcome the extra funds; but in the long-run such a shift in emphasis would not be a good thing; it might mean that we could produce more results, but it would give the administration less ability to put these results into practice," says Dr Robbins of NIOSH. □

Next week: problems with non-proliferation

Scientists leaked H-bomb secrets, says US

US Government experts believe that the authors of two press stories outlining the concepts behind the hydrogen bomb may have been helped by leaks from scientists at the government's own Lawrence Livermore and Argonne national laboratories.

The government had tried to stop publication of an article by Howard Morland entitled "The H-bomb Secret — How We Got It, Why We're Telling It" in the *Progressive* magazine. However, the government dropped its case after the publication last week of a lengthy letter outlining the details of the H-bomb in a Wisconsin newspaper, the *Madison Press Connection.*

The letter, written by a computer-programmer, Charles Hansen, has roused. the suspicions of government nuclear experts, who claim that Hansen used leaks from the government's secret files in its case against the *Progressive* in addition to repeating the mistakes made by Morland in his article. As for the latter, the government claimed in a brief that "The evidence suggests that Morland was able to write accurately about the H-bomb secret only because he had significant guidance by a person or persons with access to classfied material".

Both authors insist that they based their articles on information available to the public in libraries, lectures, and textbooks, and that the similarities in the two articles could be explained by the fact that they used the same sources.

Despite the decision to drop the *Progressive case,* the legal battles are not over, since the government has now asked the Justice Department to determine whether there is a case for criminal prosecution under the Atomic Energy Act.

But lawyers on both sides have doubts as to whether a firm case could be made. The lawyers for the *Progressive* argue that the Act is too loosely defined for a successful prosecution; and some lawyers in the Justice Department believe the government's case would be fundamentally flawed, as the many of the "secrets" are already in the public domain.

Anne Norman

Poland seeks cooperation on nutrition

Fundamental research into human nutrition, including data gathering and the design of experiments suitable for use on human subjects, should become a major area of international cooperation, Professor Kazimierz Szebiotko of the Poznan Agricultural Academy told *Nature* last week. He was attending a UK/Polish symposium on food technology at the National College of Food Technology, Weybridge.

Poland already has a well-developed programme of food research. Protein production and its optimum use rates as a "governmental problem" in the Polish hierarchy of research and development, "commanding the highest priority. The eight-point programme ranges from plant genetics to unconventional protein production, and from the biochemistry of aminoacids to the optimum animal feeds.

So far, said Professor Szebiotko, results have been generally better than expected. A soya-bean has been developed with the shorter growing period necessary for Poland, and already some 500 ha have been grown successfully. In collaboration with a Canadian team, a strain of rape-seed has been developed with very low content of erucic acid (suspected of being a carcinogen).

There have, however, been setbacks. A project for processing inconveniently small fish and fish scraps into edible form turned out well — but ran into trouble when fish stocks declined. There are certain logistic difficulties; in his own field of single-cell protein production from agricultural wastes, Professor Szebiotko said, developing the process was fairly straight forward. What was needed now was research into the "economics of cooperation with industry".

This did not seem to have been the case, incidentally, with TVP. According to Professor Jan Zaleski of the Ministry of Food Production, who led the delegation, for some years now all Polish hamburgers, mince and many kinds of sausages have contained TVP "extender" (20% in the case of hamburgers and about 12% for sausages), the new recipe being quietly introduced and the non-extended product simultaneously phased out by agreement between the Ministries of Food Production and Health — without any indication to the public. This, incidentally, was not the best procedure in Professor Szebiotko's opinion. He would have preferred the open launching of frankly soya lines.

In the future, he hoped, genetic engineering would form a fruitful field of international cooperation. Other such "themes for the future", to be tackled at the international level, he suggested, might include the biochemistry of nitrogen fixation, the optimal use of water resources (also a major concern in Poland), and his own particular interest, optimal nutrition.

So little is known about this, he stressed, while so much work has been done on malnutrition and obesity. What is needed, he suggested, is a data-bank of nutritional experience collected over the whole world, No one country, or even group of countries, could undertake this. It is a task for the United Nations agencies, for WHO and FAO.

Vera Rich

Cubans to join the space race before 1983

Cuba's future cosmonauts have received special training and acclimatization to enable them to cope with a possible landing in the Siberian winter, the chief training officer of the Interkosmos programme, former cosmonaut V. A. Shalato, told a Moscow press-conference recently, despite the fact that Soviet space planners are usually reluctant to give any details of future plans or cosmonaut training.

The press conference was, formally, a winding up of the latest series of Salyut-6 missions. The emphasis on future Cuban participation does not necessarily indicate an imminent Cuban launch. It could simply be a response to the Havana non-aligned conference and the large Latin-american presence at the press-conference.

Shalatov would not commit himself to a date for the Cuban launch, recalling only that it was originally announced that all the Comecon allies would have had a cosmonaut in orbit by 1983, and that "we have every reason to think we will meet this date, or perhaps it will be a little earlier." He did, however, say that the Cubans had completed their general theoretical work and had now moved on to crew training.

At present, the two candidates are officially on holiday in Cuba, (where incidentally, Interkosmos maintains a cosmonauts' rest home), One of them has also been taking examinations at Havana University.

Meanwhile, according to Academician Boris Petrov, Chairman of the Interkosmos council, the programme of on-board experiments proposed by the Cuban scientists for the future cosmonauts to perform is undergoing expert scrutiny by "specialists of both countries".

Vera Rich

"If they're gonna put a Cuban in space maybe we can fix it to be Fidel Castro!"

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Lake Balaton: going green

HUNGARY's Plant Protection and Agrochemical Service was originally established 25 years ago to boost agricultural production by the proper application of modern fertilizers and pesticides, a task which it carries out efficiently and well from its 20 sophisticated research centres (one in each county and one in Budapest). During the last three years, however, the centres in Zala, Veszprem and Somogy counties have taken on an additional role, the protection of the water quality of Lake Balaton. This is a task of some urgency: a recent report to the Hungarian government stated that with the present rate of chemical input, and no preventive measures, the majority of the lake will be green with eutrophication and unacceptable for leisure use within 15 years.

Lake Balaton is the largest body of fresh water in continental Europe with an area at mean water level of 595 km^2, and it is Hungary's main holiday area. In 1963, a special government decree pronounced that the region should be developed primarily to meet the needs of domestic and foreign tourism. A number of highrise hotels sprang up along the lake shore — not always with sufficient attention to effluent disposal.

The main problem, however, is agriculture in the catchment basin, an area of some 5,180 km^2, excluding the lake surface. The main crops are wheat, maize, rape and, on the northern shore, grapes. Modernisation of agriculture has led to a considerable increase in the use of agricultural chemicals. By 1975, the average concentration of active nitrogen phosphorus and potassium substances (NPK) had reached 400 kg/ha in Somogy county, 330 in Veszprem, and 380 in Zala. The daily discharge into the lake via the river Zala and other feeders was 764 kg of total phosphorus and 1,600 kg of total nitrogen from plant nutrients, with an additional 254 kg of total phosphorus and 533 kg of total nitrogen from treated effluent.

To exacerbate the problem, environmental protection in Hungary was covered by a piecemeal series of laws until as recently as 1976. These were often outdated, as, for example, a 1947 order of the Ministry of the Interior which made it an offence to encourage draught animals by shouting. In 1976, however, a new and comprehensive act came into force: "On the protection of the human environment". To implement this legislation, the former Office of the Environment was upgraded and given independent status as the State Council for the Environment and Nature Conservation, with a secretary of state, Dr Gyorgy Gonda, at its head. To deal with the specific problem of the Balaton area, a Coordinating Council for Research on Lake Balaton was established under the auspices of the Academy of Sciences, headed by Dr Istvan Lang, Deputy General Secretary of the Academy.

A complex ecological research programme was inaugurated, originally with some 200 subjects on the agenda. After what Dr Lang describes as "repeated professional consultations" these were finally whittled down to three priority research areas: factors characterising, impairing or improving water quality in the lake; the social sciences background to environmental decisions in the region; and the environmental factors affecting recreation and tourism. In addition, two longer-term studies were also given priority status: the review and synthesis of the principal scientific results and professional experience relating to the region, and the working out of the scientific basis for an integrated monitoring system.

Some 40 research institutes, university departments and industrial research laboratories were drawn into the project. Government guidelines indicated that the lake water from now on must be used for drinking and recreation only; even treated effluent is to be excluded. While civil engineers and architects are busy on a crash programme of sewerage development, the three plant protection stations in the counties bordering the lake have joined forces with the National Water Authority to monitor and prevent the run-off of chemicals.

Close to the lake and its tributaries, an upper limit of 160 kg/ha of active NPK has been set, with some pesticides and fertilizers forbidden completely. The restricted zone is normally 1 km wide, measured from the bank, with very severe restrictions south of highway 71 on the north shore of the lake. Although a reduction in fertilizer use should lead to decreased crop yield, recent rises in the price of fertilizers have raised the general question of how cost-effective it is to apply massive doses of fertilizer. Many of the erstwhile lavish cooperatives are in any case settling for slightly lower yields. One of the main research subjects for the Plant Protection and Agrochemical Service is how to increase crop-yield without increasing the amount of fertilizer used. As far as pesticides are concerned, the Plant Protection and Agrochemical Service was already operating a compulsory soil and residue monitoring service for food crops. In the three lake counties, this was extended, in 1976, to the monitoring of pesticide run-offs. After an initial survey carried out together with the National Water Authority, a set of soil and water sampling sites were chosen at random positions. These sites are monitored every fortnight or month (according to agricultural conditions). According to Dr Sandor Bordas of the Budapest Plant Protection and Agrochemical Station, it is now possible, on the basis of the last three years' results "to follow the dynamics of pollution, and to have a regular view of the quality of water and soil in relation to pesticides".

Balaton fisherman mends his nets

According to the research team at the Zalaegerszeg Plant Protection and Agrochemical Station, the new restrictions will have little overall effect on the region's agriculture. With some crops, however, there may be "difficulties". Vine-growing, in particular, is now very difficult in the restricted zone of the north shore, and the biological research institute at Szeged has started research into disease-resistant strains, which would reduce the need for pesticides. So far, however, no variety has been obtained that is entirely resistant to *Plasmopora viticola* (downy mildew). Should vinegrowers wish to restock, they explained, the government will give direct financial assistance to state or cooperative vineyards. Much of Hungary's viticulture, however, takes place in the private sector, on the small 'household plots' which provide some 30% of all Hungary's crops. Small growers wishing to make the change-over, said the Zalaegerszeg team, get 8 years free of tax on the new vineyard.

The small grower, incidentally, is potentially one of the weak links in the whole plant protection service. On cooperative and state farms, the use of agricultural chemicals is strictly controlled by specially trained Plant Protection Engineers. No such restrictions can be imposed on the private grower — other than by limiting the sale of the most sophisticated products to the state sector. Theoretically, then, there is nothing to stop the owners of summer cottages on the north shore from pouring unacceptable concentrations of fertilizer on to their gardens — except a growing public awareness with ecological problems, which is currently being promoted by a massive campaign in the media.

Vera Rich

news in brief

Pillinger wins appeal against dismissal: A UK employment appeals tribunal has ruled that Dr David Pillinger was unfairly dismissed from his post as a research biochemist at the Christie Hospital in Withington near Manchester. Pillinger, 40, had held a permanent post at the Christie since 1962 but was made redundant when the Medical Research Council failed to fund an experimental chemotherapy project he was working on (28 January). A previous tribunal ruled against Pillinger but on appeal Mr Justice Flynne found that the redundancy was "grossly unfair" since the hospital did not make an effort to find out why Pillinger's grant was denied. The hospital will now apply to the Court of Appeal for leave to appeal and if this is granted it will present its case to the Court later in the year. In the meantime Pillinger is without compensation. Even if the Court of Appeal finds in his favour he will have to face a further tribunal to obtain remuneration for the damages caused by his dismissal.

Two hundred demonstrate for Piperno in Paris: Demonstrators marched through Paris last week in support of Franco Piperno, the Italian physicist and left-wing political activist who is threatened with extradition to Italy for "activities directed against the State". Piperno has drawn support from intellectuals in both France and Italy against his extradition. In Paris, the Comité des Intellectuels pour l'Europe des Libertés (CIEL) — an organisation of leading European intellectuals headed by playwright Eugene Ionesco — issued a statement last Thursday oposing a renewed demand for Piperno's extradition by the Italian authorities. "The rules of jurisprudence are opposed to an extradition based on manifestly political motives. The Paris court refused this extradition the first time and the Italian judge, seeing the inanity of his first formulation has prepared a second dossier consisting of an improbable judicial patchwork of no less than 46 indictments." In Italy, 50 Italian intellectuals, including writer Romaine Gary and film director Bernardo Bertolucci have expressed their opposition to the arrest of Piperno in Paris and the persecutuion of other activists in the Workers Autonomy Movement, an independent political grouping to the left of the Italian Communist Party. The Paris Court of Appeal will examine the second extradition request on 26 September.

Canada announces inquiry into nuclear energy: The Canadian Minister for energy, mines and resources, Mr Ray Hnatyshyn, announced last week that the Canadian government is planning an open parliamentary inquiry into "the whole field of nuclear energy". In a speech to provincial mines ministers in Winnipeg, he said that the inquiry will be conducted by a parliamentary committee, but that the government did not intend to place any type of moratorium on the further development of nuclear power during the inquiry. Full details of the nature and scope of the inquiry are to be decided after the Canadian parliament reconvenes in October.

French unions find fault with reactor part: Two French trade union federations, the CFDT representing metallurgy workers and the CGT representing workers in the energy industries, have announced "important" faults in the fabrication of a part destined for French nuclear reactor assemblies. The two federations made their announcement in a letter to the Minister for Industry, M. Andre Giraud, on 22 September. An official inquiry has been ordered into the origin and evolution of the fault. The fault in question consists of fissures from 7 to 8 millimetres long and 6 millimetres deep in the tubular plates of the heat exchanger-steam generator, one face of which has contact with the water from the primary reactor cooling circuit. The CFDT says that with some 20 reactors affected, French on-site safety measures are not adequate to deal with the situation. The CGT while saying that there is no immediate hazard is calling upon its members not to start up the reactors without "fully guaranteed security conditions." A decision will be made at the end of the month whether to start up certain reactors or not.

US nuclear fuel factory shut down: The director of the Nuclear Regulatory Commission's safety division has ordered the shutdown of a factory that manufactures fuel for US Navy nuclear submarines. The factory was closed after an NRC inventory revealed that a significant amount of highly enriched uranium was unaccounted for. The exact amount has not been disclosed but regulations stipulate a shutdown within 72 hours if more than 19.8 pounds of uranium is missing. The Nuclear Fuels Services Inc. facility which is located in Erwin, Tennessee has had a history of problems. In April 1977, the factory was fined $53,000 by the NRC for failure to provide adequate security services. The facility will remain closed for 45 days while a complete uranium inventory is made.

US Navy tested germ warfare on San Francisco: The US Navy blanketed 117 square miles of San Francisco with an aerosol spray of bacteria in 1950 in an attempt to measure the effects of biological warfare, according to recently released military records. For six days in September 1950, a Navy ship released an aerosol containing *Serratia* sp. bacterial (thought to be harmless at the time) over the city. In spite of a subsequent outbreak of *Serratia*-related pneumonia the Navy kept quiet about the tests until last week when documents were released to the family of Edward Nevins, a victim who died during the outbreak. The family is suing the Army for $11 million. The Nevins case raises questions about 239 similar open-air experiments conducted by the Army in the twenty years between 1949 and 1969.

Telecommunications for Third World: The use of new technology to provide reliable telecommunications for remote rural areas in Third World countries was one of the few practical suggestions to be made at the recent UNCSTD meeting in Vienna. Already worked out in considerable detail and coordinated by the International Telecommunications Union in Geneva, the proposed Global Domestic Satellite System aims to make use of the increasing reliability and convenience of geostationary satellites (types such as INTELSAT 5 and SYNCOM 4 were referred to in the paper) as the central transmission facility. Such a satellite would transmit to transistorised ground stations operable on a 12-volt battery pack costing an estimated $ US 15,000. Retransmission would be by line of sight radio to the surrounding area. ITU believe that the rapid growth and refinement of the techology involved indicates the feasibility of launchings in 1982/3.

India to train Vietnamese scientists: India will train scientists from Socialist Republic of Vietnam under a programme of science and technology co-operation signed in New Delhi in July. The training will be in survey techniques, railway technology, information sciences, water resource management and research in drugs and medicinal plants. Areas of further co-operation between the two countries, in the fields of water management, pollution control, highway engineering research, traditional herbal remedies and medicinal plants will be explored by Indian scientists who will be sent to Vietnam. The programme is follow-up action of an agreement on cooperation between the two countries signed in Vietnam earlier. *Zaka Imam, New Delhi.*

Thai researchers go for mushrooms, not poppies: The Thailand Institute of Scientific and Technological Research, Bangkok, has been investigating the cultivation of the button mushroom on paddy straw, one of the major agricultural wastes of the country. It is hoped that it will be suitable for cultivation by the poor hill tribes of North Thailand — where the opium poppy presently provides much income.

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Why Britain does not need a Minister for Science

By Margaret Thatcher

Ian Lloyd, a Conservative MP, was the chairman of the sub-committee on science of the Commons Select Committee on Science and Technology. We publish excerpts from an exchange between him and Margaret Thatcher on science policy

Sorry about the Select Committee

‘**Dear Prime Minister,** I hope you will not mind my sending you two documents which bear on the exchange of views on science policy administration between us at Question Time on Thursday 12 July. The first is an article by Judy Redfearn in *Nature* (12 July, 98-99) on the role now being filled by Pierre Aigrain in France. The second is a guest leader in *New Scientist* which I have been asked to write (2 August, 346).

Pierre Aigrain was Chairman of the Committee on Information Technology in the EEC which now bears his name. He has made a distingushed and forceful contribution to science policy. The role which he now discharges in the French government is precisely what I had in mind when putting my question. Apart from yourself, no Minister, to my knowledge, has the same function here. There is no national "science budget", only a series of departmental science budgets . . . There is, therefore, no overall view and such a view is urgently required.

Aigrain has commissioned and is publishing a report on the organisation of French science and the resource allocation policy behind it which he claims, I believe rightly, to be unique. I do not know of any report which is as comprehensive as "L'état des sciences et des techniques francaises" and I am not aware of any document in the UK which provides information from which government, or anyone else interested in science policy, could draw conclusions similar to those set out in the inset article on page 99 of *Nature*.

I hold a strong conviction that we need such a Minister, such an allocation of responsibility, and that a similar report on the organisation and effectiveness of scientific research within the UK is required. I do not believe that a general sympathy for the importance of science and technology within the existing departmental structure — important though it may be — is a sufficient condition for the successful appliciation of science in the United Kingdom in today's circumstances . . .

My other reason for raising this issue is the profound dismay which I believe we have caused by a failure to re-establish the Select Committee on Science and Technology. I know that the Parliamentary and Scientific Committee is most concerned about this and that this lies behind the decision of the House of Lords to set up their own Select Committee on Science and Technology which they hope eventually to broaden into a joint committee of both Houses. While this is no substitute for the Commons committee, it will salvage something from the wreck and I strongly support their Lordship's initiative. Their contribution of scientific expertise will be most important and it is conceivable that such a joint committee, provided it has the government's suport, may well develop into an organisation around which a thorough and wide ranging review of national policy and performance in the field of science and technology policy might be built . . . ’

‘**Dear Ian,** You will remember that your point was extensively debated here in the early 1970s. In fact there was then a more centralised science budget than there is now . . . But the centralised system was not considered satisfactory.

Our system was given its present shape by the 1972 White Paper "Framework for Government Research and Development" (Cmnd 5046). . . These arrangements were reviewed by the last Government. The result of the review was published in March this year as a White Paper (Cmnd 7499).

Our system recognised that Government-sponsored applied science and technology is not an end in itself, but a means of helping to achieve the Government's policies and objectives. It follows that policy on applied science and technology in any sector should be associated with policy on investment, human resources, market needs and other factors, and should therefore be the concern of the Minister responsible for overall policy in that sector. But there is one sector — fundamental research — where there is no close link between research and policy. For that, it makes sense to entrust responsibility to the Minister who is responsible for those institutions of higher education where much of this type of research is done . . .

If we went over to a centralised system with a separate Minister for Science with his own department we would have to accept the disadvantage of divorcing those responsible for applied R and D from those concerned with formulating and implementing the policies to which their R and D related. In fundamental science we would have an unwelcome division between responsibility for higher education and for the scientific community supported by the Research Councils.

At present we have machinery to ensure that there is no harmful overlap between Departmental R and D programmes and policies, that no gaps arise, that policy questions with a major scientific or technological content are considered inderpartmentally; where this is necessary and that the quality and direction of R and D in any area, or over all areas, can be assessed. Since 1976 a committee of permanent secretaries and chief scientists has provided interdepartmental coordination of science and technology matters at high official level; and the Central Policy Review Staff play an active part in the overview aspect . . . .

I note your view that we need for Britain a report on the organisation of our science and the resources that go into it on the lines of what the French are doing. I should like to consider in due course whether such a report would be valuable . . . . The Research Councils and several Departments publish annual reports on the R and D, and it may take some time to digest the implications of the reduction in public expenditure to which we are committed. But I am quite sure that we do not need a Minister for Science to prepare such a report . . .

I am not saying that our present system should never be adapted. On the contrary, I intend to keep an eye on how it is and modify it as may be required. I recognised, for example, that under the present arrangements issues may arise which straddle the responsibility of several Ministers to such an extent that it would not be sensible to ask one of them to take the lead. In such a case I would myself play a coordinating role . . .

Personally I was very sorry that the House decided not to re-establish the former Select Committee on Science and Technology. I thought it did valuable work and was complementary to the role of departments. But you and I were overruled by the vote — however much we both regret it. ’

From begging bowl to bread basket

In his final report on science in India, Anil Agarwal describes the green revolution that has put India in the position of being able to feed its starving millions. But only in principle . . .

In 1967, William and Paul Paddock wrote a book called "Famine 1975 — America's Decision: Who Will Survive?" In this book, the Paddock brothers classified developing countries, based on their population growth rates and potential to grow food, into three categories: 'can't be saved', 'walking wounded' and 'should receive food'. India suffered the ignominy of not only being classified as 'can't be saved' but also of being accredited with the comment that it "is the bellwether that shows the path which the others, like sheep going to slaughter, are following." India was thus a country, according to these authors, to which the US should not have bothered either to provide aid or food.

It is therefore almost with glee that Dr M.S. Swaminathan, the former Director-General of the Indian Council of Agricultural Research (ICAR), seems to have quoted these statements of the Paddock brothers in his lecture to the Administrative Staff College of India at Hyderabad now entitled: "From Begging Bowl to Bread Basket". For indeed, as seen in 1979, the genetic package that India adopted in the late 1960s in the name of the green revolution, is now increasingly turning it into a major, and hopefully stable, producer of cereals. India has had bumper harvests for the last four years (1975-79). In 1978-79, the grain output touched a high of 126 million tonnes, culminating in a record government-held grain reserve of 26 million tonnes, which is more than half the total import of foodgrains by the Third World in 1975.

India has recently supplied several countries like Afghanistan, Bangladesh, Viet-Nam and USSR with food shipments and, at one point, even Pakistan, was reported to be considering a request to India for grain. A special committee appointed by the government recently recommended that India should start planning grain and vegetable exports on a regular basis to develop stable markets, though initially, it said, the grain exports could be kept at a low level of 1 million tonne a year. Some Indians still remain cautions about rushing into a venture that may not be possible to sustain. Many indeed find it difficult to believe that India can become a grain exporter. Without any doubt, the succession of good weather for four years has largely been responsible for the bumper harvests, and India's climatic pattern shows that after every 3-4 years of good weather, there is a recurrence of 1-2 years of serious drought. But, simultaneously, a feeling is growing that with the spread of irrigation, fertiliser use and high acreage under high yielding varieties (HYVs), the increases in agricultural output that India has been witnessing, are of a permanent nature.

In fact, the time when this belief will be tested is already here. Northern India is currently witnessing, according to some meterological reports, its longest dry spell since 1906. The Chief Minister of the populous northern state of Uttar Pradesh recently claimed that Rs 7000 million worth of agricultural output has been destroyed in his state alone. But Dr Swaminathan, now the Agriculture Secretary, claimed at a press conference that the fall in India's total production will certainly not be as precipitous as it used to be after drought years in the past. And, furthermore, he pointed out that the problem in 1979 is not one of grain scarcity, but one of organising efficient distribution of grains and launching crash food-for-work programmes so that the rural poor, whose crop has been destroyed, can get employment.

Even outside India, several organisations are waking up to the agricultural potential of India. The World Bank, in its annual report for 1979, released this week, claims that there are growing indications that the 'floor level' of India's grain output has been raised. James Grant, President of the Overseas Development Council, Washington, even believes that India is fast acquiring the potential not only to feed itself but also countries outside. Crop yields in India are still very low. Its acreage is very large. This gives India an enormous 'untapped yield reservoir', as agricultural scientists call it.

How to triple output of grain

In an article published in a book entitled Mobilising Technology for Development, released last month at the UNCSTD, Grant explains: "Yields per acre in India (particularly of rice, which has the largest acreage) are aproximately 1,000 pounds, compared to 3000-4000 pounds in the more advanced countries (including even Third World countries like Taiwan and South Korea). India has about the same overall quality of natural environment — land, water resources, temperature, *etc.* — as the United States; it also has roughly the same arable acreage. Twenty per cent of its land is under irrigation (in comparison to 10% in the US), and this could easily be doubled in the 1980s — at a time when worldwide additions to irrigated acreage are expected to slow sharply as compared to the past 25 years.

"India, therefore, could be producing at least three times more grain per acre than it is now doing, at present cost levels, if it could overcome certain problems of organisation, finance, and applied research. This would increase its total output from the current level of 120 million tons of grain to more than 300 million tons (compared to a present US level of 250-260 million tons)."

Indian should actually attempt to do so, argues Grant, because there is the growing need for an alternate breadbasket for the world. The International Food Policy Research Institute, Washington, has estimated that the Third World's food imports will triple to about 120-145 million tonnes every year by 1990, and this may be beyond North America's capacity to supply alone.

The success that has been achieved already is to a great extent the result of investments made in agricultural research. The ICAR was set up in 1929 by the British colonial government — but nobody even knew in 1964 "where the ICAR was in the organisation chart of the government", to quote a senior Indian science planner. It was then a small 'attached office' in the Ministry of Agriculture, totally bureaucratised and emaciated. But in that year, the ICAR was reorganised as part of the country's drive towards the green revolution, and expenditure on agricultural research by ICAR rapidly grew from Rs 64 million in 1965-66 to Rs 370 million in 1976-77 (though still less than the amounts spent on either defence, atomic energy or space research). Research is now conducted through a grid of agricultural universities, 29 central research institutes and numerous all-India coordinated research projects sponsored by the ICAR.

Indian agricultural scientists have received considerable support in their work from international institutes like the International Maize and Wheat Improvement Center in Mexico and the International Rice Research Institute in the Philippines, but they themselves have conducted substantial research to adapt imported genetic material to local environmental conditions, find new high yielding varieties (HYVs), and develop the agricultural practises required to support them. International agricultural R&D centres have now multipled to eleven, but recognising that they cannot take the role that national research organiations should play, as shown by the work of ICAR, the Consultative Group on International Agricultural Research will set up a new institution called the International Service for National Agricultural Research (ISNAR) in 1980. ISNAR will mainly help to develop national crop research centres.

But successes notwithstanding, Indian agricultural research faces even stronger challenges in the years ahead. It is important, for instance, to develop more biological sources of nitrogen fixation like blue-green algae, which reduce the need for

Joseph Hanlon

Increasing rice production by for a second crop

expensive chemical imputs. Without this type of new agricultural technology, most small and marginal farmers will remain seriously constrained in using the green revolution technology package. A large part of the increase that has taken place in rice production in recent years has been contributed by the rich farmers of Punjab, which is not a traditional rice-growing state. During 1978, the government procured more rice from Punjab alone than in the whole of the rest of India. Punjab's rice yields are more than double the all-India average yield. Rich Punjab farmers were also the forerunners of the green revolution in wheat. Punjab produced more wheat in 1978-79 than was produced in all India 1951-52. Eastern India, however, which is the traditional rice-growing region, has not benefitted equally from the new genetic package in rice. This region is dominated by extremely small landholdings and prone to floods, which submerges the short HYVs.

Major research into pest control

Pest and disease control is another major area of research. The danger of pest and disease invasion is much higher in tropical regions and HYVs have not only been, in general, found to be more susceptible compared to the traditional varieties, but their introduction has resulted in some minor pests, like the brown plant hopper, emerging as major threats. Integrated pest control and surveillance practices which reduce or obviate the need for pesticides will, therefore, have to be developed.

Protecting the "ecological infrastructure", as Dr Swaminathan puts it, is yet another area for careful research and planning. Intensification of agriculture and increased pressure on forests for domestic fuel and commercial timber, particularly in the Himalayas, is now resulting in serious floods year after year. Environmentally sound agriculture and forest managemnt in mountainous ecosystems is, therefore, of prime importance to India. But substantial research has yet to be done in this area and, even more work has to be done to introduce what is known to the local people.

Equally important is the question of soil fertility. Burning of cowdung and lack of tradition for using human excreta as manure means that organic fertilisers are not reaching the soil. In certain heavily cultivated northern states, research is now showing that even certain micronutrients like zinc are becoming constraints to increases in yields which can only be partially offset by the increased use of nitrogenous chemical fertilisers.

Research in non-cereal food also needs considerable attention. Pulses, which provide most of the protein in the Indian diet, have suffered a setback in production as farmers have increasingly turned away from pulses to grow the more remunerative HYVs of important cereals in the wake of the green revolution. Until HYVs of pulses are also available, farmers are unlikely to give them high priority. The India-based International Crops Research Institute for the Semi Arid Tropics is now actively conducting research on certain pulses. But several other minor crops that are locally important, for instance, in the hills, continue to be under-researched.

Conservation of germplasm too is a vital area. India is a rich centre for genetic variability in several important cereals, vegetables, cotton, pulses and fruit trees. With increasing deforestation and cultivation of monocultures spreading rapidly, this genetic treasurehouse stands threatened. Organisation of germplasm banks is thus of extreme importance.

Some good progress has certainly been made in research on dryland farming, largely aimed at reducing risks in cultivation in rainfed areas. Even with full untilisation of available water in the Indian subcontinent, there will be large areas that will not be able to receive assured irrigation. Even small fluctuations in the monsoon often spell disaster for farmers in rainfed areas and agriculturists in such areas are usually very poor. The ICAR has been doing extensive research in this field. It hopes eventually to provide farmers there with the option of switching over quickly to an alternate crop that can be grown in the remaining period of the crop season. This would ensure the farmer some return at least in that year.

Crop life-saving research, which aims at saving the plant in periods of acute stress, has also been showing good results, The integral part of such research is water management at the level of every watershed. Each watershed is advised to make a pond to collect the run-off and use this water to save the crop. Essentially, this is not a new advice. Most villages in ancient India have a village pond. Together with this engineering approach, efforts are also being made to develop new drought-resistant HYVs.

Vicious effects

The future of agricultural research in India is thus both bright and challenging. But whether all Indians will benefit from it equally is something that will depend much on India's social and political will. The green revolution technology has had a vicious effect on the rural poor. But to what extent the new agricultural technology is responsible for it, is not yet clear. In a highly stratified socioeconomic environment, it is more likely that any new technology which offers major productive oportunities, will be used by the more powerful to become still more powerful.

This is true, in fact, of even the age-old technology of irrigation. "Few people seem to realise that, normally, in the case of any irrigation scheme, out of every rupee worth of direct benefits accruing from irrigation, 54 paise would go to the large and big farmers who account for only 10 per cent of the total rural households", says a report recently prepared by a senior official in the Ministry of Agriculture.

Agricultural science, however, has clearly shown that it has the potential to feed India's population, and given political will and mobilisation of the people, even provide employment on a massive scale. But will the agricultural scientists get full support in their venture? It is interesting to note that after the Pokhran nuclear explosion in 1974, the government of India had quickly announced that nearly half a dozen nuclear scientists would get the Padma Shri, India's national award. But few scientists were so honoured for their work on HYVs of wheat, which changed India's agricultural future. □

correspondence

Berufsverbot: physics professor sacked

SIR, Professor Jens Scheer, one of the leading critics of Germany's nuclear energy program, is to be forced to leave his life-long tenured position as professor of nuclear physics at the University of Bremen. The Administrative Court of the Free Hanse City of Bremen imposed this sentance of Berufsverbot (profession forbidden) in June after a disciplinary trial and retrial lasting two years. In its ruling the three-judge panel concluded: "As an appropriate disciplinary action for this offence only the heaviest one can be considered: expulsion from office".

The "offence" appears in the Federal Constitutional Law for Civil Servants. It is only because the German civil service includes, *inter alia*, permanent university staff that professorScheer is bound by this law. This circumstance has important and unavoidable consequences especially for those who must teach and carry out objective research. The court stated:

"It is expected of a civil servant that he recognises and acknowledges the State and its constitution as a high postitive value worthwhile to defend actively; Federal Constitutional Law (Beschluss von 22,5, NJW 1975, 1641)". (This is applied to one's private life as well.) "According to this law (Art. 33 Abs. 466) a civil servant stands in public-legal relations of service and loyalty. That is why it is his duty to be loyal toward the State and the constitution (political loyalty-duty of the civil servant)."

The ideal of this law has been recurrently expressed in the last century of German History as a means to bring academics under the political scrutiny of the State authority. The present authorities have not changed history.

The nature of the trials of Professor Scheer is typical of the political trials going on in Germany today on a large scale. This latest Berufsverbot trial against him was based solely political principles and membership in the KPD (Communist Party of Germany) which is a legal party. The court noted that he "prominently", "publicly" and "actively" agreed with the principles of the party (i.e. he had recently stood as a local and parliamentary candidate and had sold the party newspaper). These were the only accusations used for the conviction and sentence. He was not allowed a complete defence.

Banning may come to those not quite bound by the golden chain of civil servant status, without court proceedings, after an investigation by the secret Federal Constitution Protection Police (BVSP) or because of accusations collected from paid informants of from fellow-teachers and pupils.

Over the past five years the authorities have brought at least seven prosecutions against Professor Scheer, one after the other. Since all these attempts failed, the authorities came eventually to rely on exclusively political accusations.

The present trials is in fact one of a series of Berufsverbot trials, where new charges have constantly been laid. The trials began before Professor Scheer became politically 'prominent'.

People who support Professor Scheer or who are terrified by the existence once again of these laws are asking the Senate of Bremen to overturn the court's ruling. These people include the Rector of the University, at least three-quarters of the professors, a similar majority of the University Staff Assembly and many students.

There is no doubt "that the West German authorities disregard individual rights and use improper methods in order to silence their critics". There is no doubt that the Civil Servants Law and its reflection in Bremen are tools to that end. And there is no doubt that neither truth nor democracy can proceed on the basis of loyalty, discipline and obedience.

Yours faithfully,

UWE LAHL
W THIEMANN

University of Bremen

Indian science

SIR, "India: the struggle for useful science" by Anil Agarwal (23 August p 625) was objective — but gave very little account of the role of scientists in general. Though India boasts a large reservoir of talented and well-trained scientists, unemployment and under-employment among scientists are considerable. Many talented scientists fail to make any mark because of their inabilities to go along with the establishment. And when these scientists go abroad to prove their abilities, the establishment itself honours them. At least three Indian scientists have committed suicide out of sheer frustration in not being able to cope with the powers that be. Although everybody appreciates the role of science, no one has yet given any serious thought to the exact role and ambition of scientists in the research and developmental projects of which he is considered to be a part. Research institutions are mostly modelled on a bureaucratic pattern and, as correctly pointed out in the article, unless one turns into an administrator, a professional scientist is rarely honoured. Fortunately, there is no restriction as yet to free expression by scientists: hence this letter.

Yours faithfully,

B BANERJEE

Tocklai Experimental Station, Jorhat

Humane farming

SIR, Baker and Manwell (23 August, p630) strongly infer that I oppose "humane" treatment of farm animals by advocating intensive husbandry. This is incorrect. I am not an animal behaviourist, but I have supervised the rearing of farm animals in confinement, under experimental conditions, for many years. There are, broadly speaking, two approaches to humane treatment, pragmatic and anthropomorphic. The pragmatic approach says that inhumane treatment is counter-productive, because unhappy animals do not eat well, and hence do not thrive. Solitary confinement shows this. Chickens search frantically for a companion. In one instance, two cows would not eat when separated, but started again when a window was cut in the partition. However, companionship may be *de trop*; egg production in hens is diminished by sexual harassment by male chauvinist roosters, which are therefore kept away, except in breeding flocks.

The floor space per animal allotted under confined conditions must be adjusted for maximum productivity, which is diminished by overcrowding. Composition and intake of food must be nutritionally optimal, drinking water always available, and ambient temperature such as to be compatible with good health. Dairy cows are notoriously sensitive, not to intensive husbandry, but to minor annoyances, which may make them refuse to let their milk down.

The anthropomorphic approach that decries crowding of farm animals is of dubious validity, in view of the fact tht human beings pay large sums to be jammed together at football games and in theatres. The most successful cocktail parties are tightly packed gatherings where the noise is deafening, and hotels in the United States display signs that warn against over-occupancy of meeting rooms. The chicken that has to roam in the out-of-doors probably would choose the protection of the hen-house against foxes, coyotes and *Accipiter* hawks.

Indeed, with rare exceptions, such as myself, wilderness conservationists dislike the solitude of the wilderness, for I have noted that most devotees of the Sierra Nevada seek out the few crowded areas, such as the John Muir Trail and the summit of Mount Whitney, leaving the choicer spots to darkness and to me.

Yours faithfully,

THOMAS H JUKES

Berkeley, California

Costs of toxicity testing

SIR, Dr Conning in his letter of 2 August (p 354) draws attention to the problems facing the chemical industry worldwide, of society demanding greater assurance against the risks of error or of inadequate knowledge than it did in the past. One of the principal difficulties has been to anticipate the possible effects of a chemical on man and the environment before it was introduced into commerce, expecially in those cases where the effect may become apparent only after a long latent period.

In a Discussion Document published in June 1977, the Health and Safety Commission proposed to require by statutory regulation the formal prior study and reporting of the toxic properties of substances which were to be newly introduced. This was an attempt to establish a first order of priorities, since although — as Dr Conning rightly points out — even long experience of manufacture and use does not provide an infallible safeguard, at least by tackling urgently the 'new' substances we should not be adding to the existing list of 'don't knows'. HSE does not intend to produce guidelines on how to test chemicals, but merely to indicate what data would be needed as basic information for an evaluation of the properties of such substances.

It is common ground in this field that what we need are properly validated test procedures whereby chemicals may be screened effectively and at minimum cost in order to identify the hazards which they might present in manufacture and use. Such procedures must, however, be kept flexible in order not to dissipate resources in needless evaluation of effects which could be assessed from existing data either published or held in firms' archives.

Yours faithfully,

E W LANGLEY

Health and Safety Executive, London

news and views

Joining immunoglobulin genes

from W. Dunnick

ONE classical idea in immunology is the joining of variable region genes to constant region genes. The carboxy terminal part (constant, or C region) of immunoglobulin polypeptides is sequence invariant, and is probably coded by one or a few genes. For example, 95% of all mouse light chains have a single 'kappa' C region sequence; the remaining 5% are divided between two 'lambda' C region sequences. These protein sequences are probably encoded by a single kappa C gene and two lambda C genes. The amino terminal part (variable, or V region) of immunoglobulins is highly diverse in sequence; duplicate V sequences are rare among the hundred or so mouse kappa proteins studied to date. Fifty to one thousand genes are thought to code for mouse kappa V regions. Dreyer and Bennett proposed that the immunoglobulin 'gene' is formed from these two different gene pools by translocation of DNA (*Proc. natn. Acad. Sci. U.S.A.* **54**, 864; 1965). Thus, one V gene (out of hundreds) is joined to one C gene to form the genetic unit which is transcribed and translated.

This idea was proved correct by restriction analysis of DNA (Hozumi & Tonegawa *Proc. natn. Acad. Sci. U.S.A.* **73**, 3628; 1976), and by cloning and sequencing experiments (Bernard, Hozumi & Tonegawa *Cell* **15**, 1133; 1978). Immunologists were surprised to learn that, in the germline, the last one-eighth of the V region was not attached to the rest of the V region (where it belonged), but was closely associated with the C gene. In embryonic DNA, the DNA segment coding for the first 95 amino acids of the lambda V region was separated, by more than 4.5 kb, from the segment of DNA which included the coding sequence for the last 13 amino acids of the lambda V region (the J segment), the coding sequence for the lambda C region, and the 1,200 bp intervening sequence between J and C. In DNA of a cell producing a lambda light chain, the V segment coding amino acids 1–95 is joined to the J segment coding for amino acids 96–108 to form the contiguous V gene. The intervening sequence remaining between V and C is probably removed by RNA splicing (see *News and Views* **276**, 322; 1978, for more background and references). In the mouse, the kappa light chain system is more complicated than the lambda system; there are about 100 times more kappa V segments than lambda V segments. The analysis of kappa J segments by Max *et al.* (*Proc. natn. Acad. Sci. U.S.A.* **76**, 3450; 1979) and by Sakano *et al.* (*Nature* **280**, 288; 1979) has revealed a system slightly more complicated than the single lambda J segment, but also more informative.

Both groups cloned a segment of embryonic DNA that included the kappa C gene and 4.5 kb upstream from the C gene. A large part of the DNA was sequenced, and five J segments were identified by homology to kappa protein sequences. The J segments are in a cluster, separated from each other by about 300 bp; the nearest to the C gene is about 2.5 kb away (Fig. 1). In the 5′ direction, there are no additional J segments within 1.1 kb; there are probably none between J5 and the kappa C gene. The data cannot exclude additional J segments elsewhere. From other data it seems unlikely, though not impossible, that additional kappa J segments exist.

J segments and joining

What recognition signals are important in V–J joining? Adjacent to the 3′ end of sequenced V coding segments and to the 5′ end of each of the five J segments is the palindromic sequence (or a slight variant of) C–A–C($^{A}_{T}$)G–T–G. This sequence is also found following a lambda V segment and before the lambda J segment (Bernard *et al. op. cit.*). A second consensus sequence is found 25–30 nucleotides from the J segments, G–G–T–T–T–T–T–G–T–A. The homology is so striking and well-preserved that one feels the presumptive joining enzyme would recognise these sequences. Using the cloned V and J segments, one might begin a search for the joining enzyme. This could be a formidable task, as no one knows in what cells V–J joining takes place, or how many of these cells can be isolated.

Of the five J segments, examples of four can be found in known kappa protein sequences. The J3 sequence is not found in any known protein. It has the most divergent sequence of the set, and the intervening sequence following J3 does not begin with G–T, even though almost all other intervening sequences do (Breathnach *et al. Proc. natn. Acad. Sci. U.S.A.* **75**, 4853; 1978). Thus, Max *et al.*

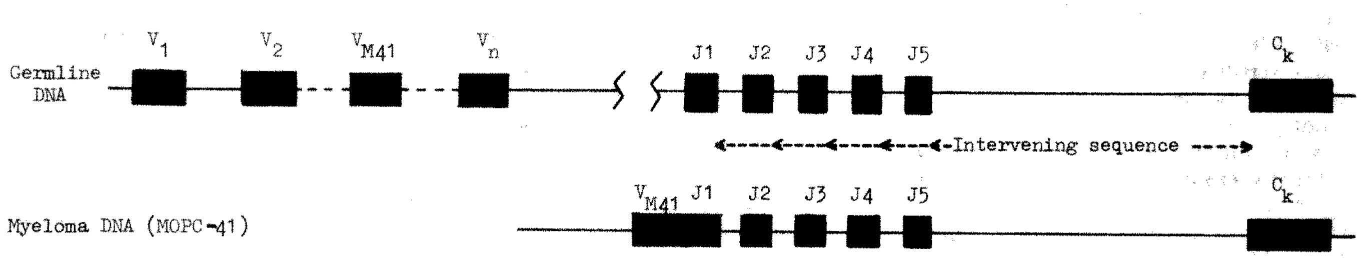

Fig. 1 Arrangement of kappa light chain genes in germline and myeloma DNA. Adapted from Seidman *et al.* (*Nature* **280**, 370; 1979). This figure is not drawn to scale. Darkened boxes are coding sequences. The exact arrangement of the V segments is not known, relative to other kappa V segments or to the kappa J segment and C gene.

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suggest that J3 is not expressed in protein because its intervening sequence cannot be properly excised. The correlation between expression and junction sequence is interesting, but there is a second exception to the G–T rule which is certainly excised properly and expressed (Sakano *et al.* *Nature* **277**, 627; 1979). How rigid is the G–T rule? In the J3 segment, is divergence away from G–T at the beginning of the intervening sequence a cause for non-expression or a result of non-expression?

J segments and diversity

Both Max *et al.* and Sakano *et al.* have noted that V–J joining may generate sequence diversity. Bernard *et al.* (*op. cit.*) and Weigert *et al.* (*Nature* **276**, 785; 1978) have also suggested the possibility. By adjusting the point of recombination within the three nucleotides following the V coding sequence and the three nucleotides of residue 96 in the five J segments, one can generate all known residue 96 amino acids in Balb/c and NZB kappa proteins. As an example, the nucleotide sequence at the end of the MOPC-41 V segment is (Seidman *et al.* *Nature*, **280**, 370; 1979):

Pro 95| non-coding DNA
-C -C -T -C -C -C -

The first three nucleotides of J1 are -T–G–G–. Recombination joining the first C after the V coding segment to the two Gs in the J1 segment yields -C–G–G–. C–G–G codes for arginine, residue 96 in MOPC-173 (residues 97-108 of the MOPC-173 kappa chain are consistent with the sequences of J1). Other possible results of recombination are -C–C–C- (Pro, MOPC-11), -C–C–G- (Pro, MOPC-11), and -T–G–G-(Trp, MOPC-41). Another possible event joins the three Cs following the V segment to the three nucleotides coding for residue 96 in the J segment. For example, joining -C–C–C- following the MOPC-41 V segment to -T–A–C- at the beginning of J2 yields Pro–Tyr, the residues 96 and 96a of 7132, a NZB kappa chain which has an 'extra' J amino acid.

Does sequence diversity at residue 96 lead to new antigen combining sites? This question, though central to discussions of diversity, is difficult to answer. In the few immunoglobulins whose three-dimensional structure is known, amino acid 96 is near or at the antigen combining site (Padlan *et al.* *Immunochemistry* **13**, 945; 1976). It is probably safe to assume that diversity generated by V–J joining, in some cases at least, can alter the antigen binding properties of immunoglobulins.

V–J joining is an exciting topic because it is the first example of a defined molecular mechanism which probably leads to V region sequence diversity. This is a step forward for immunology. Nevertheless, V region diversity must also be generated by other mechanisms. None of the sequence differences in mouse lambda V regions can be explained by V–J joining. In mouse kappa V regions, 20–30 amino acid residues show more sequence variability than does residue 96. The light chain of myeloma-2413 (from a NZB mouse) has aspartic acid at residue 103 and glutamic acid at 107. Both residues are lysines in all five Balb/c germline J segments. Either the NZB has at least one different J segment, or somatic mutation must act even on J segments.

From work during the past three years, culminating in the papers by Sakano, Max and their colleagues, we know a great deal about light chain J segments in the mouse genome. Protein sequence data suggests that heavy chains also have J segments (Rao *et al.* *Proc. natn. Acad. Sci. U.S.A.* **76**, 2890; 1979), and recombination has been postulated to occur near residues 96–104 in human kappa chains (Milstein *Nature* **216**, 330; 1967). Several questions concerning J segments will be answered by studies in these areas. How many J segments do the kappa and lambda systems have in humans, where the two light chains are about equally represented in serum immunoglobulin? Does each heavy chain C gene have its own J cluster? Does the role of J segments in diversity extend beyond V–J recombinations? □

W. Dunnick is a postdoctoral fellow at the MRC Laboratory of Molecular Biology, Cambridge, UK.

Sun shines on photosynthetic prokaryotes

from A. J. Smith

THE clear message to emerge from recent studies with photosynthetic prokaryotes is that there is much more to the microbial world than *Escherichia coli.* Of particular interest is the application of recombinant DNA techniques to the analysis of the genetics of these organisms. At a recent symposium* B. Marrs (Saint Louis University, Missouri) described how a promiscuous plasmid with sex factor activity has been used to mobilise segments of the chromosome of *Rhodopseudomonas capsulata* including that carrying the genes for photopigment synthesis, reaction centres and tryptophan synthetase. The cloning of portions of this DNA for use in studies of gene structure, function and regulation is now possible following the isolation of R-prime plasmids in which the photopigment genes are incorporated into the plasmid structure. The genetic analysis of cyanobacteria has been handicapped by difficulties in the isolation of mutants and the lack of efficient systems for gene transfer other than transformation which has been demonstrated in a number of strains. R. Haselkorn (University of Chicago) has avoided these problems in an investigation of the genes for nitrogen fixation (*nif* genes) in an *Anabaena* sp. He identified fragments of cyanobacterial DNA containing *nif* genes by hybridisation with recombinant plasmids containing defined segments of the *nif* region of *Klebsiella* DNA and found a 10 kb fragment which contained sequences complementary to *Klebsiella* genes *D*, *H* and *V* (genes *D* and *H* code for components of nitrogenase). Sequences complementary to genes *Q*, *B*, *A*, *F*, *M* or *S* were not found in any of the DNA fragments, suggesting that the *Anabaena* counterparts of these genes have diverged too far to be studied by this technique. The discovery of closed covalent circular DNA (plasmids) in a variety of cyanobacteria (G. A. van Arkel, State University, Utrecht; F. Doolittle, Dalhousie University, Halifax, Nova Scotia; V. Saunders, Liverpool Polytechnic), has introduced the possibility of using them to mobilise sections of the chromosome of these organisms. The Utrecht group reported the successful incorporation of an ampicillin-resistance marker of the *E. coli* plasmid pR146 into a small 53,000 molecular weight plasmid in *Anacystis nidulans* R-2. In the gene transfer system they are currently developing, this modified plasmid will be used as the cloning vehicle for fragments of chromosomal DNA by the technique of insertional inactivation. This insertion of the complete sequence of the Tn*901* transposon into the cyanobacterial plasmid along with the ampicillin resistance marker may yield a new method for producing mutants if the jumping character of the DNA segment is preserved in its new location. These recent advances will open the way for analyses of genes specifying the photosynthetic apparatus, nitrogen fixation and the mechanisms for their regulation as well as the characterisation of processes governing the development and differentiation of photosynthetic prokaryotes.

Photosynthetic bacteria and cyanobacteria include organisms which are being used as model systems for the study of morphogenesis and differentiation as indicated by the review papers on *Rhodomicrobium vannielii* (C. Dow, University of Warwick) and cyanobacteria (C. P. Wolk, Michigan State University). Factors determining the production and spacing of heterocysts, which are the sites of nitrogen fixation in certain filamentous cyanobacteria, have yet to be fully characterised. Furthermore, the identity of the reductant that passes from vegetative cells to the heterocyst to support nitrogen fixation and the origin of glutamic acid which is used to export combined nitrogen in the opposite direction have yet to be established with certainty. Reports of reproducible procedures for the production and germination of akinetes (M.

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*The Third International Symposium on Photosynthetic Prokaryotes was held on 27-31 August in Oxford and was organised by N. G. Carr (University of Liverpool.)

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Herdman, Dundee University) which are a form of non-growing resistant cyanobacterial cell indicate that the analysis of this further example of prokaryote differentiation is now possible.

Recent work on pigment organisation and primary photochemistry which was reviewed by P. Thornber (University of California, Los Angeles) highlighted the specialised features in some photosynthetic prokaryotes of the antenna complexes which harvest light energy and channel it into the photosynthetic reaction centres. Chlorosomes (formerly known as *Chlorobium* vesicles) which fulfill this function in green photosynthetic bacteria consist of a relatively unorganised assemblage of bacteriochlorophyll *c* molecules in a non-unit membrane-bound sack positioned in close proximity to bacteriochlorophyll *a* – protein complexes in the cytoplasmic membrane. The antenna complexes of cyanobacteria and red algae are known as phycobilisomes. D. Bryant (Pasteur Institute, Paris) proposed that these highly organised complexes of several types of phycobiliprotein consist of a triangular core of allophycocyanin molecules with three peripheral rods radiating from each of two sides of the triangular core, the rods being made up of phycocyanin and of phycoerythrin. The sharing of certain carriers between photosynthetic and respiratory electron transport which has been demonstrated in photosynthetic bacteria may prove to be a feature of cyanobacteria as well; the dark aerobic utilisation of molecular hydrogen by *Anabaena cylindrica* is likely to involve the transport of electrons along the section of the carrier chain containing plastocyanin and plastoquinone (H. Bothe, University of Cologne).

The factors that play a critical part in establishing the dominance, albeit transient in some cases, of natural populations of photosynthetic prokaryotes are ill defined. Recent field and laboratory studies have introduced the real possibility of interpreting ecological phenomena in terms of the physiology and biochemistry of photosynthetic prokaryotes. H. van Gemerden (University of Groningen) proposed that the availability of acetate is a factor likely to contribute to the appearance of blooms of *Chlorobium phaeobacteroides* in Lake Kinneret. Other reports attributed the survival of a large population of the cyanobacterium *Oscillatoria limnetica* in the 'solar lake' in Israel despite extreme and rapid fluctuations in conditions to the metabolic flexibility of this organism. Besides the ability to switch from anoxygenic to oxygenic photosynthesis (B. B. Jorgensen, Hebrew University, Jerusalem), this organism is also able to catabolise endogenous polysaccharide reserves either by an aerobic respiratory mechanism or by a lactate yielding fermentation under anerobic conditions (M. Shilo, Hebrew University, Jerusalem).

In the session on evolution R. E. Dickerson (California Institute of Technology, Pasadena) maintained a lively hope that protein sequence studies would, in due time, yield data of phylogenetic significance, the accumulation of new information serving to minimise the blurring effect of lateral gene transfer. This final session closed with a progress report delivered in inimitable style by R. Lewin (La Jolla, California) on the characterisation of prochlorophytes which are associated with colonies of didemnid ascidians. Their seemingly opposed affinities on the one hand to bacteria in terms of ultrastructure and on the other to green algae in terms of their photosynthetic apparatus has been further confirmed by work in various laboratories. The outstanding requirement here is for conditions to be identified which will transform these ecological eccentrics into creatures amenable to laboratory investigation. □

A. J. Smith is in the Department of Biochemistry and Agricultural Biochemistry, University College of Wales, Aberystwyth.

Photosynthesis research in the USSR

from D. O. Hall

In the USSR a massive effort is being put into photosynthesis research in all its various aspects. To improve crop yields, physiological and agronomic aspects must be understood; if biomass is to be used as a source of energy photosynthetic efficiencies must be improved; and if photosynthesis is ever to be mimicked to provide 'futuristic' energy, for example hydrogen and fixed carbon and nitrogen, the basic processes of photosynthesis must be understood. All these aspects of research into photosynthetic mechanisms are being undertaken in the USSR in a seemingly determined way.

A travel exchange between the Royal Society and the USSR Academy of Sciences recently enabled me to visit three centres of photosynthesis research — the Moscow-Pushchino region in Russia, Tashkent in Uzbekistan, and Kiev in the Ukraine, all of whose research has some relevance to solar energy utilisation. The huge Biological Centre at Pushchino incorporates the Institute of Photosynthesis, which to my knowledge is the only such Institute in the world devoted solely to the study of photosynthesis. A new young director Y. E. Erokhin was recently appointed and has responsibility for several hundred research workers and technicians in the departmental groupings — photochemistry, biochemistry, molecular organisation and structure and physiology. The many laboratories specialise in most aspects of photosynthesis, from whole plants down to biophysical work on pigments and membrane structure.

The first report of a thermostable hydrogenase (from the red photosynthetic bacterium *Thiocapsa roseopersicina*) emanated from I. N. Gogotov's laboratory at Pushchino. They showed that this hydrogenase had a temperature optimum of 75 °C and was quite stable in air — before this hydrogenases were thought to be very unstable. Now a range of hydrogenases have been reported from various organisms which are reasonably stable and are thus useful in biophotolytic systems which evolve hydrogen gas. This is an active area of research in Gogotov's laboratory as it is part of the Soviet Union's solar energy programme.

Phytotrons, computer facilities, and special equipment services go to make up a very impressive Institute. A detailed report on its activities will soon be available in English. Two other Institutes in Pushchino — those of Biochemistry and Physiology of Microorganisms, and of Protein Research usefully complement the photosynthesis research groups.

In Moscow the Academy's Institute of Biochemistry houses the Laboratory of Photobiochemistry under Academician A. A. Krasnovsky (of Krasnovsky reaction fame — the photochemical reduction of low potential compounds using artificial chlorophyll systems). Krasnovsky's laboratory is studying hydrogen-producing systems using model pigment systems, for example, chlorophylls, TiO_2 particles, viologens, ferredoxins, hydrogenases and platinum. They were the first to show in the 1960s that TiO_2, ZnO and WO_3 could split water with ultraviolet light, evolving oxygen and reducing ferric and quinone compounds. Work continues in placing such systems in micelles and liposomes and on multilayers. At Moscow State University the Laboratory of Chemical Enzymology in Berezin's Chemistry Department is also looking at biological energy converting systems.

Work is progressing on trying to stabilise chloroplast membranes (not yet totally successful, as everyone is finding out) and on growing algae in ponds for subsequent fermentation to methane. They also have an active group on the construction of fuel cells using hydrogenases immobilised on gels and graphite electrodes; an oxidase, for example, laccase, comprises the other half cell. Such fuel cells seem quite stable and operate at high efficiencies.

In Skulachev's Laboratory of Bioenergetics model membranes as photoconvertors are an active area of research. In the Department of Microbiology, Professor E. Kondratieva heads a famous group working on C, H and N metabolism of photosynthetic bacteria and on methane-producing bacteria. The growth of photosynthetic bacteria under dark and light conditions and under various oxygen

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D. O. Hall is Professor in the Department of Plant Sciences, King's College, London.

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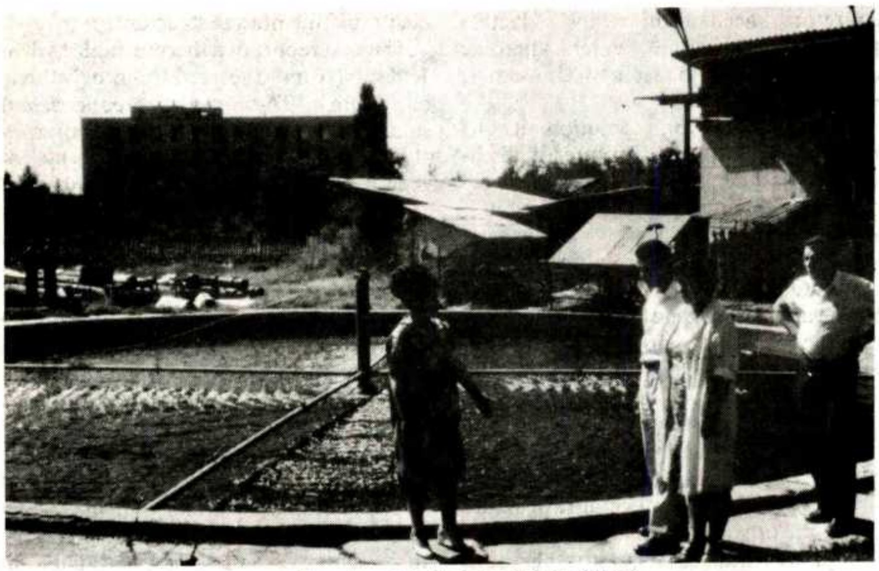

Experimental algal pond in Tashkent

tensions has an important bearing on their ability to fix N_2 and evolve H_2 — besides using various carbon compounds as a substrate source. This laboratory has shown that *Thiocapsa* can be grown chemoautotrophically in the dark under oxygen but it needs thiosulphate and CO_2 — it cannot use organic compounds under such conditions. This relates to Pfennig's work in Göttingen on the non-sulphur *Rhodopseudomonas* which can grow in the dark with H_2 and CO_2 when oxygen concentrations are low. These observations seem important since they point to an adaptability of photosynthetic bacteria not hitherto realised. Kondratieva's laboratory is also actively involved in the possible utilisation of photosynthetic microorganisms in solar energy conversion systems.

In the Academy's Institute of Plant Physiology in Moscow, Professor A. A. Nickiporovich heads the Laboratory of Photosynthesis which has a long history of work on factors determining the efficiency of photosynthesis and plant productivity. Nickiporovich heads the group in the USSR Solar Energy Committee looking at biomass as a future source of energy, while, Krasnovsky leads the group investigating the potential of photosynthetic mechanisms as solar energy convectors.

The Republic of Uzbekistan has over 400 algal ponds in operation today. It was an eye-opener to visit the Institute of Microbiology at Tashkent headed by Academician A. M. Muzofarov and run by the Uzbekistan Academy of Sciences in Tashkent. Besides practical research in mycology (the first volume of floral mycology of Uzbekistan having just been published) and fermentative microorganisms, there is a significant effort on algal ecology, physiology and productivity.

Ponds growing green algae under thermophilic and mesophilic conditions apparently operate in many parts of the Republic to provide food supplements for cattle, chickens and silkworms. Yields vary from 30 to 60 tonnes dry weight per hectare per annum, the limiting factor being low winter temperatures of 2 – 5 °C although there is still considerable sunshine. A large system near Samarkand produces a tonne of wet cells per day while much larger ponds are under design. Interesting experiments are also being done with blue-green algae, with water plants like *Lemna* and with fertilisation of cotton with algal suspensions.

At the Ukrainian Academy's Institute of Plant Physiology at Kiev, Professor Grodzinsky heads a team of 400 people who work on various aspects of the agronomy, nutrition, physiology, biochemistry, chemistry and physics of plant growth. Two of the eleven science departments — physiology and ecology, and biochemistry — are directly concerned with photosynthesis. Optimum conditions for productive growth under ideal and stress conditions have been analysed to help in plant selection and agricultural programmes of the Ukraine — which is the largest producer of sugar beet in the world. The chloroplast membranes and their photosystem particles and lipids are being actively studied to try to understand the inherent stability of such membranes in light and also how they might be used in artificial water splitting systems for solar energy conversion.

In Professor Ostrovskaya's laboratory there is an active group studying the localisation in and isolation of photosystems I and II from the chloroplast membranes; it seems that for photosystem II, proteins are more important for stability than the lipid environment. A photosystem II fraction has been isolated which has no contaminating photosystem I particles — this is important for spectroscopic studies.

In Professor Gulaev's laboratory they are looking at the factors limiting photosynthesis in the leaf. Seven factors can be listed and their theoretical and practical interactions are being studied. Such practical work is important in trying to select plants for optimum field growth and also under artificial conditions such as greenhouses where CO_2 fertilisation and waste heat, from power stations for example, can be used to maximum effect.

Many other centres of photosynthesis research exist in this vast country. Three of which I am aware are the Botanical Institutes in Leningrad in Russia, Baku in Azerbaijan, and Minsk in Byelorussia and there must be many more smaller photosynthesis laboratories. My impression is that photosynthesis research has been recognised as being important to the future agricultural, ecological and solar energy prospects of the USSR. ☐

Galactic X-ray sources

from A. N. Parmar and P. A. Lamb

ASTRONOMERS are becoming increasingly aware that to understand X-ray sources better they must observe them over the entire electromagnetic spectrum. Participants at a recent multidisciplinary meeting* presented the results of observational and theoretical studies ranging from the radio to the gamma-ray region of the spectrum.

The evolutionary model for a massive binary X-ray source begins as a 20 $M_\odot$ and a 8 $M_\odot$ star. The more massive star evolves to fill its Roche lobe and transfers its hydrogen atmosphere to the secondary, which becames the more massive component. At this stage the system consists of a massive hydrogen and a helium star, which rapidly collapses and probably explodes as a supernova, leaving behind a neutron star. The hydrogen star becomes a supergiant with a strong stellar wind, providing materal which accretes onto the neutron star: this powers an X-ray source for about 5 million years.

There are many problems with such a simple model. Mass conservation is assumed, although it is known that many such stars are over-luminous for their masses, which implies that they lose mass at a high rate. The effect of mass loss on stellar evolution is not at all clear but some preliminary results were given by E. P. J. van den Heuvel (Amsterdam). The model does not explain the origin of the slow pulsars (with periods of order of a minute) although some mechanisms to slow down newborn, rapidly spinning neutron stars (which have periods of fractions of a second) have been proposed.

Several contributors demonstrated how observations over a particular wavelength range can contribute to our understanding of X-ray sources. The search for radio emission from X-ray sources was covered by B. Burk (Massachusetts Institute of Technogy). In a typical binary system undergoing mass exchange X rays are

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*An Advanced Study Institute on Galactic X-ray sources, was held at Cape Zunion on 28 May-9 June, and sponsored by NATO, The Greek National Committee for Astronomy and University College London's Department of Physics and Astronomy.

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often produced in the high density, high temperature, accreting plasma close to a compact star while the radio emission is formed further out by the interaction of the relativistic plasma and the magnetic fields that are generally present. The well known X-ray binaries, Cyg X-1, Cyg X-3, Cir X-1 and Sco X-1 as well as GX17+2, the Galactic Centre and various RSCVn stars have been detected at radio frequencies.

The optical characteristics of high mass binary systems were discussed by J. B. Hutching of the Dominion Astrophysics Observatory (DAO). Detailed spectroscopic studies of eight of the hot supergiant components indicate that although these primary stars are undermassive (compared with normal stars of the same spectral type) their elemental constituents are apparently normal. The transfer of mass is made evident by the emission lines at λ4684 (He II) and the $\lambda\lambda$4640 — 4650 (C III — N III) blend. Both emissions almost certainly occur in the accreting matter.

Analysis of optical light curves for these systems is complicated by the need to correct for non-circular orbits, tidal deformation, X-ray heating, non-synchronous rotation of the primary and the presence of an accretion disk.

A. Cowley (University of Michigan) presented a review of optical investigation of low mass binary X-ray systems. These fall broadly into three groups: first, those in which the optical output from the disk dominates that from the 'normal' star. The optical spectrum then consists of a hot, blue, relatively featureless continum with λ4686 He II emission. An example of such a system is Sco X-1 for which spectroscopic radial velocity measurements imply an orbital period of 0.782 days. However, no eclipse (or pulse) periodicity has been detected in the X-ray data. The second type of system is where the optical emission is dominated by the 'normal' star, for example Her X-1 and Cyg X-2. The latter system contains a low mass population II F star which fills its Roche lobe. A 9.8 day orbital period has been determined for this system by radial velocity measurements. The third type of low mass systems are the cataclysmic variables. These consist of the AM Her types (with strong emission lines and magnetic fields), SS Cyg type systems (common dwarf novae) and some of the X-ray transients (for example AO620-00).

A. K. Dupree of the Smithsonian Astrophysical Observatory (SAO) reviewed recent IUE and Copernicus observations of X-ray binaries. Such observations can lead to a better understanding of many problems such as the interaction between X rays and a radiatively driven stellar wind. High temperature material in an accretion disk or X-ray heated photosphere can also be observed in the ultraviolet. Changes in the ultraviolet spectrum of Vela X-1 may be caused by X-ray photoionisation and heating of the stellar wind. The effect of X rays on the interstellar medium has been detected in observations of both this source and 4U1700-37. Two narrow, highly excited, C IV and Si IV interstellar lines have been detected with absorption features separated by 50 km s^{-1}. These may be produced by the expansion of a 'bubble' of ionisation which expands as the X-ray source photoionises the interstellar medium. Ultraviolet analysis of the chromospheric emission from many of the hot single stars, which are now being found to be X-ray emitters, is an important new means for investigating the intriguing problem of the formation of the coronae.

Observations using the HEAO-1 and Einstein satellites have identified new classes of X-ray emitting objects and made possible a more comprehensive survey of known types of source. The emission of X rays from RSCVn, flare and main sequence stars has now been verified. About 80 discrete sources can be seen in the nearby galaxy M31, whereas the last generation of X-ray instruments are only able to detect the whole galaxy as a single source.

J. Grindlay (SAO) reported recent results on the enigmatic X-ray sources seen in some globular clusters. With the increased positional resolution of the Einstein Observatory it has been possible to measure the offset of the X-ray source from the centre of the optical cluster with precision. From an assumption that the stars in the core have an isothermal velocity dispersion, he has calculated the mean mass of this class of X-ray source. This turns out to be of order 20 $M_\odot$. There is, however, a large error associated with this value (2 $M_\odot$ is allowed for with a confidence of 10%) owing to the uncertainty in position of the optical centre of each cluster.

A hundred years ago

ONE feature of the last eruption of the remarkable volcano of Kilauea, in the Sandwich Islands, is the fact that the great molten lake of lava, occupying a hugh caldron nearly a mile in width, and known as the "South Lake," was drawn off subterraneously, giving no warning of its movements and leaving no visible indication of its pathway or the place of its final deposit. "Other eruptions," writes Dr. Coan to Prof. Dana, in a letter dated June 20, "have blazed their way on the surface to the sea, or while on their subterranean way have rent the superincumbent beds, throwing out jets of steam or of sulphurous gases, with here and there small patches or broad areas of lava. But as yet no surface-marks of this kind reveal the silent, solemn course of this burning river. One theory is that it flowed deep in subterranean fissures, and finally disembogued far out at sea.

From *Nature* **20**, 25 September, 513; 1879.

Previous Copernicus results which have tenatively identified OAO1653-40 with the massive binary V861 Sco were supported by more recent data from the same satellite which was presented by A. Parmar (MSSL). This showed an ultraviolet burst, which in one C III line alone had a luminosity of 2×10^{37} ergs s^{-1}, occurring simultaneously with an X-ray turn on. If it can be shown that V861 Sco is indeed associated with the X-ray source then it becomes a firm candidate for a black hole of mass between 7 and 13 $M_\odot$.

H. Bradt (MIT) described the detection of rapid aperiodic variability in Galactic X-ray sources. This has long been known to characterise Cyg X-1 and Cir X-1 although, with the recent detection of pronounced flaring and millisecond bursts, it also seems that the time variability of these sources is significantly more complicated than was once realised. In the past year, rapid aperiodic variability has been observed from GX301-2 and GX339-4. A 7 to 8 second delay between the arrival times of the optical and X-ray signals from Sco X-1 has been interpreted as the light travel time from the compact body to the primary. Correlated optical and X-ray activity has been found by S. Ilovaisky (Observatoire de Paris, Meudon), when the source flares.

Seven of the 16 known X-ray pulsars have had their orbits accurately measured by Doppler analysis of their varying pulse periods. One source, which may be a system containing a neutron star and a low mass white dwarf is 4U1626-67. S. Rappaport (MIT) reported on the search for orbital motion and the detection of as yet unexplained quasi-periodic bursts with a 1000 s characteristic timescale, which could be caused by material in unstable orbits which decay onto the neutron star.

Rappaport has combined data from the Ariel 5 and SAS-C satellites, and obtained a revised orbit for GX301-2 (4U1223-62) which has been determined with a most probable period of 40.8 days and a non-zero eccentricity. Optical photometry on the other hand suggests a period of about 20 days. Vela X-1 has been shown to have an eccentric orbit. Future observations of the change in the longitude of periastron will enable the amount of central condensation of the primary star to be calculated.

Detailed analysis of the X rays detected during an optical flare from the dwarf nova SS Cyg undertaken by F. Cordova (Caltech) shows the 9 s pulses to have a very sinusoidal shape. However, the pulse phase is unstable which makes a determination of the orbit very difficult. Similar

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A. N. Parmar and P. A. Lamb are at the Mullard Space Science Laboratory, University College London.

Problems in hominid taxonomy

We are pleased to have had our recent systematic assessment of hominid evolution recognised in *Nature* (*News & Views*, **278**, 400; 1979). Your Paleoanthropology Correspondent has, however, provided the readers of *Nature* with a misleading analysis of our published work as well as a misunderstanding of systematic procedures followed therein.

Your correspondent was not familiar with the authorship of the formal designation of the new taxon, *Australopithecus afarensis*, Johanson, White and Coppens, 1978 (not Johanson and White as reported). The correspondent also seems unaware of the difference between diagnosis and description in a systematic publication. Features of the dentition (large mandibular canines, three-rooted upper premolars and molar size gradients) and postcranium (lack of third metacarpal styloid process, os *coxaae* anatomy) taken by your correspondent as diagnostic of *A. afarensis* are detailed in the *description* of the species and not the *diagnosis*. Of the suite of characters distinguishing *A. afarensis*, the correspondent mentions only a few dental features and completely neglects diagnostic cranial and mandibular anatomy.

Unfortunately, treatment of our *Science* article (**203**, 321; 1979) fares no better as several errors, in fact, mar the report. For instance, the correspondent reports that the Taung specimen does not have a P$_4$ but that we measured it. In fact, the specimen has two P$_4$s, but we did not measure them because they are unerupted. Similarly, our table of dental metrics (page 322) shows central incisor measurements for five individuals instead of the two reported by your correspondent. The correspondent also states that the *A. afarensis* P$_3$ "has a large outer cusp and a very small inner cusp". The relevant citation from *Science* (page 322) is "A smaller lingual cusp is usually present, but some specimens (A.L. 288-1, 128-23) display only an inflated lingual ridge." The fact that this feature reminds the correspondent of Miocene hominoid P$_3$s emphasises our assertion that the CP$_3$ complex of *A. afarensis* is an important character suite that allies the new taxon with extant and extinct apes and distingushes it from members of other hominid species.

Finally, the correspondent suggests that variation (not variability) in the Hadar sample is excessive and indicative of taxic diversity. We direct the reader to our *Science* paper where all of our metric and morphological comparisons of the Hadar and Laetolil samples with those of extant hominoids support the interpretation that the fossil hominid material is best considered a single, naturally variable, sexually dimorphic taxon.

Nature's policy during the controversy over the status of the Taung skull was "No notice is taken of anonymous communications". (*Nature* **116**, 462; 1925) We favour that forgotten policy.

Donald Johanson
Laboratory of Physical Anthropology
The Cleveland Museum of Natural History, Cleveland, Ohio.

Tim D. White
Department of Anthropology,
University of California,
Berkeley, California.

Our Correspondent replies: While I thank Johanson and White for their response to my recent News and Views comment I must point out that their reply does not effectively refute any of the major points of that comment. One of the major premises of that comment was that the diagnosis of the new species (Johanson, White & Coppens *Kirtlandia*, **28**, 1; 1978) does not adequately differentiate it from *A. africanus*, the closest comparative taxon. If that premise can be substantiated then the validity of *A. afarensis*, as a separate and distinct taxon, can be questioned. The necessity of a clearly stated differential diagnosis was noted by the authors when they quoted Mayr's imperative to "list the most important characters or character combinations that are peculiar to the giver taxon and by which it can be differentiated from other similar or closely related ones" (*op. cit.*, page 8). Diagnoses in the general palaeontological literature show that most workers follow Mayr's dictum. Yet precise differential statements are missing from this diagnosis; those features which qualify *A. afarensis* as a separate species have not been made clear.

Moreover, characters which may have a high degree of taxonomic significance have been described differently in the original diagnosis and in a later publication. The size of the inner cusp of the *A. afarensis* P$_3$ is a case in point. In the diagnosis of the new species it was stated that this feature "is often expressed only as (an) inflated lingual ridge (*op. cit.*, page 6). In a later publication the "often expressed" ridge had become a "usually present" cusp (Johanson & White *Science* **203**, 322; 1979). They cannot have it both ways and the fact that they have described the same important character state differently in two publications does not add strength to their taxonomic claims.

Further confusion in their work is apparent when, in their response, they claim that five individuals were used in obtaining measurements of the upper first incisor. Reference to my comment will show that part of that discussion centred on their claim that *A. afarensis* had broad upper first incisors. The breadth of this tooth is measured in the mesiodistal orientation and reference to their Table 1 (*Science* **203**, 322; 1979) will show that four teeth were measured in this orientation, not five individuals (presumably yielding 10 teeth as they now inexplicably claim.

Moreover, Johanson and White suggest that I have confused, in my comment, the diagnosis of the new species with its description. Again, it is they who are confused. That stated that "certain traits . . .(were) placed in the description due to the lack of comparative anatomical specimens from other species of *Australopithecus*". (*Kirtlandia* **28** 8; 1979). In my comment I pointed out that in characters of the hand, wrist and os coxae not only do comparative specimens exist for *A. africanus* but they are virtually identical in the two species.

The second major point of the News and Views comment did not concern the validity of the new taxon but centred instead on the number of taxa which may be contained in it. The authors acknowledge that the metric and morphological ranges may be broad in *A. afarensis* but they claim in the response that "all" of their comparisons between the ranges in the fossil sample and in living hominoids have supported attribution of the fossil material to a single taxon. They do indeed present (in footnotes) coefficients of variation for three features demonstrating that, in selected features, the range of variation in *A. afarensis* does not exceed that of living hominoids. Figure 6 which purportedly "clearly" (*Science* **203**, 329, footnote 23) compares the range of variation for one *A. afarensis* feature with that of other hominoids is, in fact, an illustration of two distal femora.

In the end, however, the *A. afarensis* sample is an extremely important one and disputes over its taxonomic placement do not minimise that importance. Johanson and White are to be praised for their rapid publication of this material.

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phenomena, described by T. Chester (Caltech) have been observed from another dwarf nova U Gem. He presented a technique for modelling these variations which preserves the phase of harmonics. Improved upper limits for higher harmonics of the pulses can be extracted.

The gas flow from a primary star to a compact body is complicated and poorly understood, making the interpretation of X-ray spectra very difficult. Spectral features from atomic transitions in iron and other elements have been observed in galactic X-ray sources. With the advent of more sensitive spectrometers, such as on the Einstein satellite, many more spectral features are being discovered. R. McCray (Colorado) showed how the analysis of spectral features will allow a determination of the physical conditions in, for example, the flow of accreting material near a compact object. The 'standard model' (appropriate to the solar corona) of an optically thin plasma, in collisional equilibrium, does not hold near to a compact body or in a supernova remnant. An alternative model, which is similar to that for a planetary nebula, is a central point X-ray source which photoionises its surroundings into highly excited states, even at low temperatures. Here, the Auger effect and fluorescence will be very important and radiative recombination lines may dominate the spectrum.

N. Shakura (Sternberg Astronomical Institute) has calculated the characteristic scale of turbulence in a disk which leads to an estimate of α (the ratio of the stress to the pressure in the outer part of a disk). His result, $\alpha \sim 10^{-3}$, differs significantly from previous estimates. Simulations using the precessing, twisted accretion disk model proposed to explain the 35 day cycle of Her X-1 which were presented by J. Petterson (University of Illinois) require α to be of order unity. This is not necessarily inconsistent with Shakura's work as α may vary throughout the disk. □

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review article

Contact inhibition and malignancy

M. Abercrombie*

Strangeways Research Laboratory, Worts' Causeway, Cambridge, UK

The role of contact inhibition in influencing the behaviour of malignant cells is discussed in a review. Although tissue culture cannot simulate the immense complexity of the conditions in vivo, *some of the distinctive features of malignant invasion can be conveniently observed with this technique. The evidence derived from this technique indicates that defective contact inhibition of movement of malignant cells does contribute to their invasiveness.*

COMPONENTS of the locomotor machinery of cells have been intensively studied, and a coherent hypothesis about how cells move is being developed. In contrast, little work has been done to characterise the locomotor behaviour of cells, principally the conditions that determine their direction of movement. This is in part because direct observation of the cells is only possible in the artificial conditions of cell culture. In particular, little attention has been paid to the aberrations of behaviour of malignant cells which can be observed in culture in terms of invasion, and which can be confidently predicted. In the context of the incoherent picture that unanalysed cell movement presents at first glance, the movement of malignant cells may not seem in any way distinctive. It may also be that the interpretation I have given to the invasive aspects of malignant behaviour *in vitro*, in terms of defective (not necessarily absent) contact inhibition, has given rise to doubts because it has seemed over-simplified. Nevertheless in my opinion the cardinal points remain, that invasion—the distinctive feature of malignant behaviour—can be observed in cell culture where normal cells do not invade and that a contribution to malignant invasion seems to be made by a defect in normal contact inhibition. These are the points I wish to discuss: first, how the extent of invasion may be assessed with various tumour types in culture and second, how their invasiveness compares with defects in the contact inhibition reactions of the various tumour cells.

Patterns of invasion in culture

The method we have used for assessing invasion involves confronted cultures[1] consisting of two foci of cells (explants) placed about 1 mm apart. Various measurements and observations can be made after the outgrowths have met. In our technique explants of normal and malignant sarcoma fibroblasts have been confronted with a standardised chick heart fibroblast explant, which has proved best for several reasons[2]; the choice in any case must be arbitrary. Six tumours have been studied in detail—mouse sarcomas 311, BAS56, S180, FS9, MClM and a mouse melanoma. The following observations have been made on cultures fixed 24 h after fusion.

Normal mouse muscle fibroblasts confronted with the standard chick heart fibroblasts show a very high degree of obstruction, the invasion zone (the strip occupied by cells from both explants) having a mean width of only 20 μm as shown in Fig. 1*a*. By contrast, confronting each of the six tumours with the standard chick fibroblasts resulted in invasion zones between 130 and 290 μm wide, as is illustrated for BAS56 and MClM in Fig. 1*b*, *c*. Nevertheless, there was still some obstruction of invasion in three of these tumours (311, BAS56 and mouse melanoma), as indicated by consideration of the between-to-side ratios (the ratio, for each explant, between the distance migrated towards the other explant and the mean distance migrated from its free sides). In these three cases, the ratios were less than unity for both the fibroblasts and tumour cells. The cells of S180, the tumour on which our original measurements were made[3] (but which in our experience is indistinguishable from S37 and has perhaps become confused with it) were peculiar in this respect. The between-to-side ratio was unity for both fibroblasts and S180 sarcoma cells, indicating that invasion was completely unobstructed. Nevertheless, film analysis reveals some slight obstruction of S180 cells at the fibroblast front[4] as will be described later.

The assessment of obstruction in the remaining two tumours, FS9 and MClM, is complicated by their non-reciprocal behaviour when confronted with the standard chick fibroblasts. This is best illustrated using the invasion index[2], a measure of the distance of infiltration standardised for the outward velocity from the explant which can, of course, be used separately for both cell types and which takes a value of zero when a cell type is totally stopped by the other and 1.0 when it is totally unimpeded by the other. For 311, BAS56, mouse melanoma and S180 the invasion index was approximately the same for the tumour cells and fibroblasts in each experiment (reciprocal invasiveness) but varied from 0.2 to 1.0 according to the tumour. For FS9 and MClM, however, the extent of invasion of the fibroblasts was markedly less than that of the sarcoma cells (non-reciprocal

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* This article contains editorial changes which were made by his colleague after the death of Michael Abercrombie on 28 May 1979. Reprint requests and correspondence should be addressed to Dr G. A. Dunn, Strangeways Research Lab., Worts' Causeway, Cambridge, UK.

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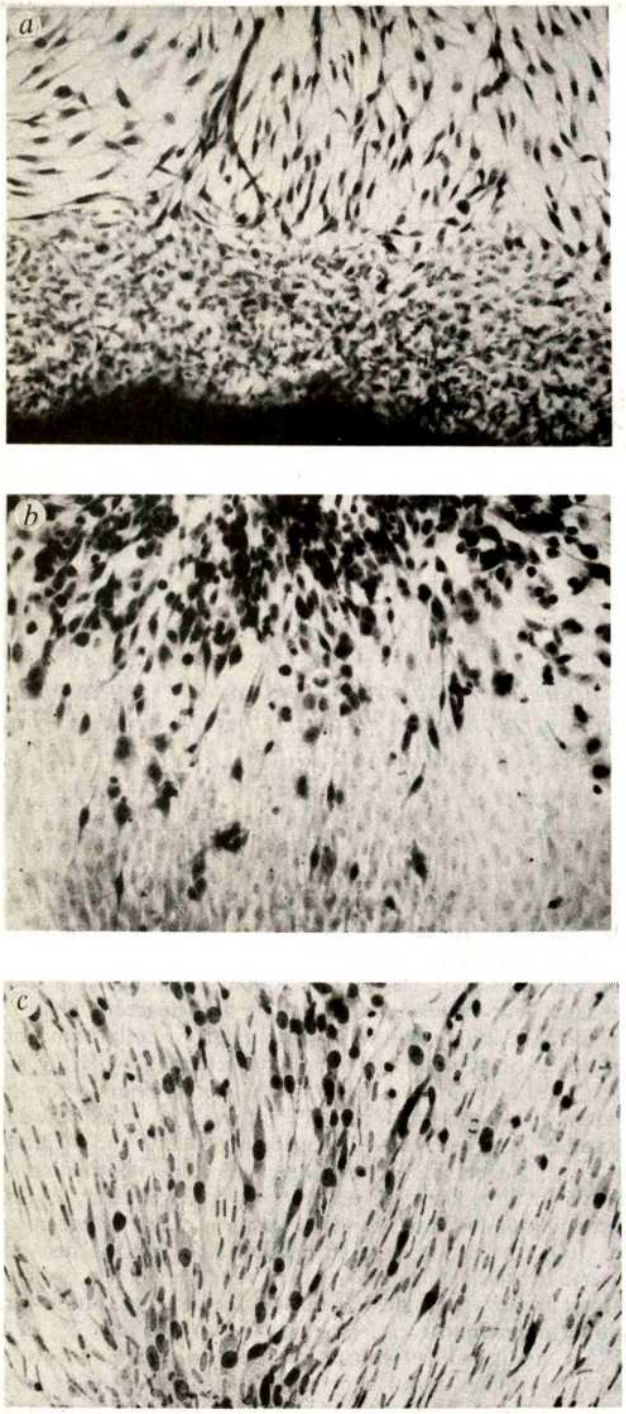

Fig. 1 Confronted explants fixed 24 h after fusion showing the invasion zone. In each case chick heart fibroblasts have migrated from below towards: *a*, Mouse muscle fibroblasts (darker nuclei)—an example of very low invasiveness, ×80. *b*, Mouse BAS56 sarcoma cells (large dark nuclei)—an example of reciprocal invasiveness, ×80. *c*, Mouse MClM sarcoma cells (large dark nuclei)—an example of non-reciprocal invasiveness which also shows the effect of contact guidance (see text), ×122.

invasiveness). The fibroblasts either get out of the way or are overrun. In FS9 the sarcoma invasion index was 0.6 and the fibroblast 0.1, and in MClM the sarcoma index was 1.5 (the sarcoma cell velocity actually increased after contact with the fibroblasts because of contact guidance) and the fibroblast index was 0.2. In these two cases, the actual distance invaded by the fibroblasts was one-third to one-quarter of that of the sarcoma cells, in spite of the fact that the outward velocities of the fibroblasts exceeded those of the sarcoma cells.

Contact inhibition

The term contact inhibition refers to the phenomenon of a cell ceasing to continue moving in the same direction after contact with another cell. It is not always operataive. To assess the part played by contact inhibition in invasion in culture we have to determine the obstruction to movement of the invading cells produced by contact with the normal fibroblasts. A useful 'static' measure of contact inhibition is the overlap index, which expresses the observed number of overlaps of cell nuclei as a proportion of the number to be expected if the nuclei were distributed at random. A homotypic overlap index is concerned with one kind of cell only, but of more immediate interest is the heterotypic overlap index, which is provided by the invasion zone of confronted populations. In this case obstruction is indicated when the number of nuclear overlaps of cells of the two different types is diminished compared with the random expectation of these overlaps[3,5]. It is necessary to score nuclear rather than cytoplasmic overlaps because of the faintness and irregular outline of the cytoplasm. Since it is a requirement that the overlaps result from cell locomotion, inaccuracies in quantification can arise from the clumping of cells due to the drawing together of contractile processes (to be distinguished from the cyclic phenomenon of true locomotion) or from the occurrence of mitosis in dense cultures where the cells cannot move away. Furthermore, the presence of even small amounts of collagen between cells can reduce the effectiveness of their mutual contact and give rise to anomalous results.

Any deviation from a random distribution of freely moving cells towards a more evenly spaced distribution (that is, any overlap index of less than 100%) means that obstruction has occurred. But a single heterotypic overlap index is obtained from the number of overlaps between two cell types and, since non-reciprocal contact inhibition has been recognised, it has become clear that one of the two cell types may be obstructed much more than the other. Thus the index of overlapping may provide a distorted picture of the behaviour of the mixed culture.

A method of obtaining a 'dynamic' index from observations of living cells has been developed for detailed analysis. It necessitates classifying the results of collisions between the two types of cell as observed in timelapse filming. Although this overcomes the limitations of the heterotypic overlap index, only a limited sample of collisions can be observed in reasonable time, particularly since clearcut collisions have to be selected. Contact inhibition is observed as one or both colliding cells stopping and perhaps immediately turning aside. Stopping will also occur at random, without collision, and therefore the excess has to be estimated. At what point does the stopping occur after contact? As seen in plan view it does not usually occur immediately after cytoplasmic contact has been made. Indeed, homotypic encounters in fibroblasts commonly show a considerable degree of cytoplasmic overlap and there may even on occasion be nuclear overlapping before contact inhibition[6,7].

It is useful at this stage to mention a confusion that has arisen over the terms overlapping and underlapping. The overlapping so far referred to consists simply of the state of superimposition of one nucleus on another, regardless of whether the upper or lower cell, by its movement, produced the superimposition (upper and lower are themselves ambiguous terms with reference to gravity) and also regardless of which type of cell is above or below when two cell types are involved. Unfortunately the state of overlapping has been confused with the acts of overlapping and underlapping. It is perhaps best to refer to one cell as either moving outside another cell, meaning on the side exposed to the medium, or moving between another cell and the substratum. From the point of view of contact inhibition all that matters is that the cells should make contact—the form of contact is irrelevant. Bell[8] has made a careful analysis of the origin of homotypic overlapping (crisscrossing) of Py 3T3 cells and found that the overlapping results from the movement of one cell between another cell and the substratum. Since no estimate of contact was made, however, it is not clear how this observation leads him to call into serious question the hypothesis that tumour cells are invasive by virtue of having lost the capacity to undergo contact inhibition.

Contact inhibition in non-malignant fibroblasts

I now consider the normal background of contact inhibition amongst non-malignant fibroblasts in culture. Abercrombie and Heaysman[1] first examined the question by measuring the homotypic overlap index in cultures of two explants of chick heart fibroblasts fixed after they had reached confluence. Several other investigations using this static method were subsequently reported (see, for example, refs 9, 10). The dynamic method of time-lapse filming for examining homotypic contact inhibition was used non-quantitatively by Abercrombie and Ambrose[11] and then quantitatively by Veseley and Weiss[12] and by Guelstein *et al.*[13]. Heterotypic estimations with mouse muscle fibroblasts confronted with chick heart fibroblasts formed the controls for the similar work on sarcoma cell invasion already referred to[2]. Whenever the static method has been applied to fibroblasts in a population that was not too dense, the number of overlaps observed has been very significantly lower than the statistical random expectation. This lack of overlapping is often referred to as monolayering.

It is evident, therefore, that contact inhibition is a general feature of fibroblast collision. But can it totally obstruct cell movement? Steinberg and his colleagues (see for example, ref. 14) have investigated this question in dense cultures of chick embryo fibroblasts, partly because of my own somewhat exaggerated claims which assumed that the displacements observed were largely in the form of oscillations. (There is in fact more mobility than that, many fibroblasts interchanging their positions at a speed of several micrometres per hour.) Steinberg's purpose has been, however, to establish the existence of motility in confluent monolayers—from which he concludes that obstruction is not total—rather than to make comparisons with motility in sparse populations or with migration from explants into cell-free space. Measurements by Abercrombie and Heaysman[15], Gail and Boone[16] and others show that when such comparisons are made, obstruction is clearly apparent.

The differential adhesion hypothesis of Steinberg and colleagues on contact inhibition amongst fibroblasts is, in my opinion, untenable as it stands. They suppose that what is inhibited is the act of passing outside and that this inhibition is due to a failure of the cell migrating outside to make effective adhesion to the dorsal surface of the cell it contacts. Typically, however, contact inhibition in fibroblasts seems to occur to a cell after it begins to pass between another cell and the substratum. Such a cell therefore undergoes contact inhibition without ever leaving its normal substratum and therefore without changing its adhesion[16,17]. Furthermore, one of the most conspicuous features of contact inhibition in fibroblasts is the contraction undergone by the cell's front end, an event which is not accounted for by the adhesion hypothesis. A notable instance of such contraction occurs in non-reciprocal contact inhibition, where the cell that is not inhibited proceeds on its way while the cell that is inhibited (if contact is made by its leading edge) retracts, leaving the way clear in front of the first cell.

The hypothesis of differential adhesion as an explanation of contact inhibition should not, however, be discarded. It may well be important in cells other than fibroblasts[4,12]. Even for fibroblasts, there might be an effect of a lack of adhesiveness of the dorsal surface which is masked by the contractile reaction. There are difficulties in testing whether the dorsal surfaces of fibroblasts are defective in adhesion independently of the contractile reaction. The scattering of a population of dissociated fibroblasts onto the surface of fibroblasts already adherent to the substratum fails to differentiate between the two hypotheses since the contractile reaction may prevent the dissociated cells from spreading as they settle.

Contact inhibition in malignant fibroblasts

I now turn to consider the relation of contact inhibition to the behaviour of malignant fibroblasts.

In the experiments we have made with explants in confronted cultures, the contact inhibition between homotypic cells within any one population has an important role. The tendency of contact-inhibited cells to move towards cell-free space produced outward radial migration from an explant; non-malignant cells with high contact inhibition migrate radially with a high velocity. Most malignant cells studied also show the effect, although since their contact inhibition is commonly lower than that of non-malignant cells they do not usually show the outward drive so powerfully. Indeed, with S180 cells we have found the outward drive to be negligible. For example, a sample of cells in an S180 outgrowth showed no significant preponderance of movements away from the explant over movements towards the explant[4]. Their motility is therefore almost entirely diffusion-like in spite of their high contact inhibition, at least at relatively low density. The explanation is probably that in contrast to the other tumours investigated the majority of changes in direction of S180 cells are spontaneous rather than the result of collision between cells. This is to be expected since S180 cells are more often out of contact with each other than the other tumour cells at the same density and they show a comparatively low persistence in direction when moving freely. E. M. Stephenson (personal communication) has recently found that the outward drive is also ineffective in mouse melanoma cultures. Although homotypic contact inhibition of the melanoma cells, judging by nuclear overlaps, is also high there is only a small but statistically significant excess of movements away from rather than towards the focus.

Although much has been made of homotypic overlapping amongst transformed cells as an index of their malignancy, the correlation, if any, is by no means clear. Most observations have been qualitative. Projan and Tanneberger[10] however, made an extensive quantitative study of tumours by means of the overlap index and found a broad range of indices from well within the normal range, to an extremely high level (2%–91%). This was in contrast to the indices of non-malignant cells which they found were concentrated within the range 3%–19%. It seems evident, therefore, that malignant cells are characterised by their broad variability as compared with normal cells, although their median level is no doubt distinctly higher. It should be added that it is not clear how a lowered homotypic contact inhibition of malignant cells could contribute to their invasiveness.

The high degree of obstruction between two confronted normal fibroblast explants, such as between chick and mouse fibroblasts, apparently results from their high heterotypic contact inhibition[2]. In a free outwandering the fibroblasts move at approximately 300 μm per day, while in confronted culture they are brought almost to a standstill. How far then, can the invasion of sarcoma cells in confronted culture be accounted for by defects in their heterotypic contact inhibition?

In sarcomas 311 and BAS56 and the melanoma, the between-to-side ratios indicate some obstruction of invasion and there is a relatively low heterotypic overlap index indicating the presence of contact inhibition[2] (melanoma data from E. M. Stephenson, personal communication), although both the extents of invasion, and the overlap indices, are higher than with two confronted normal fibroblast explants. There is no indication of any non-reciprocal inhibition, and the invasion is approximately mutual. Furthermore there is a characteristic diversion of cells at the junction of the two populations, both cell types turning in an orthogonal direction, which is probably a manifestation of their tendency to move towards cell-free space. It seems likely that because of the incidence of heterotypic contact inhibition, the outward drive from the sarcoma explant is largely arrested by the fibroblast obstruction, invasion being by a diffusion-like movement.

In S180 the between-to-side ratios gave no indication whatever of obstruction and, correspondingly, there was no deviation in heterotypic overlap from random expectation[3]. Later studies by film[4,11] showed a complete absence of contraction and paralysis of the ruffled membrane on contact on the part of either sarcoma cell or fibroblast. The films showed, however, a slight slowing down of the sarcoma cells while they were amongst the fibroblasts and a slight obstruction where they met the free edge of the fibroblast population. An unusual aspect of the invasion of S180 is that the sarcoma cells are almost invariably outside (on the dorsal surface of) the fibroblasts, which may well be a consequence of the fact that the sarcoma cells are rounded and project very much further above the substratum than do the flattened fibroblasts. There is no mutual diversion of the two populations.

In contrast to the three tumours (311, BAS56 and the mouse melanoma) which showed reciprocal invasion, two others, FS9 and MClM, showed non-reciprocal invasion. Significantly, these two tumours showed non-reciprocal contact inhibition. In FS9, the invasion indices revealed that the fibroblasts were almost totally obstructed whereas the tumour cells were only partially obstructed and, when similar cultures were studied by direct filming (Abercrombie and Turner, unpublished), the fibroblasts seemed totally inhibited on contact with the sarcoma cells though the sarcoma cells were not completely inhibited by the fibroblasts. The fibroblasts show immediate retraction on contact with the sarcoma cells when in head-on collision, while the sarcoma cells continue their locomotion. When a sarcoma cell collides with the side of a fibroblast, the fibroblast generally shows a high degree of contact inhibition; consequently, almost all the invaders are sarcoma cells. For MClM, the other example of non-reciprocity, the invasion indices show that the sarcoma cells are probably not obstructed at all. In direct filming[18] non-reciprocity is clearly seen; the fibroblasts to some extent move out of the way of the sarcoma cells after retraction, and to some extent are overlapped by them. Hence the heterotypic overlap index is relatively low even though heterotypic contact inhibition is absent or greatly reduced in the sarcoma cells. (The static measurements of overlapping can thus fail to detect some instances of failure of contact inhibition.) For this tumour the mutual diversion of cells observed with the cells of other tumours is not detectable. For both FS9 and MClM the outward drive from the sarcoma explant clearly persists into the fibroblast explant's zone (Fig. 1*c*).

Increased heterotypic overlapping in other tumours has been demonstrated in a mouse ascites carcinoma and in a mouse melanoma (by overlap index[9]) and a rat sarcoma (by filming[12]). Guelstein *et al.*[13], however, found no increased heterotypic overlapping by filming in a mouse transformed cell line.

Conclusions

It is easy to describe invasion in culture but less easy to be certain of its relevance to invasion *in vivo*. This is all the more so because there is no quantative measure of invasion *in vivo*; one has to depend on histological descriptions. Furthermore it could be that a number of transformed cells are erroneously assumed to be malignant, thus obscuring a possibly strong correlation between *in vitro* and *in vivo* invasive behaviour. It seems more probable however that 'begign' and 'malignant' are relative terms, spanning a continuous gradation of invasiveness.

Neither is it easy to account for the behaviour responsible for invasion in culture. I have concentrated on some of the complexities of contact inhibition of movement and their correlation with the overall pattern of invasion. An important outcome is the concept of non-reciprocity, formulated originally by Heaysman[18]. Its simplest interpretation requires the presence of separate emitters and receptors. Where there is no contact inhibition in either of the contacting cells, neither would have emitters or receptors for each other; where there is reciprocal inhibition, both cells would have both. The emitters and receptors must be assumed to be constant in a standard fibroblast but would differ independently as between the various cells. A tumour like MClM, which is able to induce contraction of a fibroblast—a sign of contact inhibition—would be regarded as a strong emitter, and a weak receptor, because it is not itself inhibited by the fibroblast. A tumour like S180, producing no contraction of contracted fibroblasts, would be a weak emitter and weak receptor, and one like FS9 a strong emitter but intermediate receptor. It would be instructive to test different kinds of tumour against each other, since different kinds of fibroblasts seem to vary very little in their emitters and receptors.

In addition to contact inhibition, variable adhesiveness might play a part in determining invasiveness. There may be two effects here, a homotypic effect in which cells of the same type adhere closely to each other and so prevent penetration, and a heterotypic effect in which the invaders experience obstruction because of their strong adhesion to the invaded cells. In both cases these effects are to be distinguished from the relatively low adhesiveness which is the basis of Steinberg's hypothesis of contact inhibition. The contribution to obstruction of these two possible effects of high adhesiveness cannot be accurately determined. The hypothesis that tumour cells have a low adhesiveness has been current since the time of Coman[19]. It may be said, however, that since adhesion is by definition reciprocal, the non-reciprocity found in some tumour invasions is not accounted for.

Other factors probably play a minor part in invasiveness. For instance, contact guidance by cells is incompatible with contact inhibition, but when contact inhibition is low, it is possible that the tumour cells may be guided by the underlying normal cells. Indications that this may be happening have been found in filmed S180 invasions[4]. With tumours (311 and MClM) the possible effect of some diffusable agent has been detected, there being a slight inhibition of the outgrowth of the fibroblasts before junction with the tumour cells.

The study of invasion in culture provides an opportunity for investigating the many facets of invasiveness but many important gaps remain. There has been comparatively little work on the behaviour of normal cells, and the invasion of carcinomas has not been studied at all. It should be possible to approach the *in vivo* environment of cells by using three-dimensional hydrated collagen gels as substrates. The question of the role of mitotic pressure in invasion is not suited to investigations by tissue culture. In spite of the fact that so much work was devoted in the 1920s and 1930s to the supposed growth of cultures there was never any evidence that growth as opposed to migration played any part whatever. Cells cannot be pushed along a solid substratum to which they are adhering.

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articles

Molecular abundances in IRC+10216

E. M. McCabe & R. Connon Smith
Astronomy Centre, University of Sussex, Falmer, Brighton, UK

R. E. S. Clegg
Department of Astronomy, University of Texas, Austin, Texas 78712

The observed molecular column-densities in IRC + 10216 can be matched by chemical equilibrium calculations for $T \sim 1{,}250$ K, $P \sim 100$ dyn cm^{-2}, and no graphite grain formation. Condensation of silicon carbide into grains may explain the low observed abundances of SiO and SiS. A compressed gaseous shell may be formed between successive dust shells lost by the central variable carbon star during each of its cycles.

THE IR object IRC+10216 is a barely visible, cool carbon star surrounded by an expanding, dusty molecular envelope[1-3]. Parameters for carbon stars are poorly known but[4] $R_* \simeq 1{,}000\,R_\odot$, $T_* \simeq 2{,}200$ K, $L \simeq 10^4 L_\odot$. There are grains close to the surface of the star at around 1.5 R_* from the photosphere[5]. The inner optically thick IR component[6] has a radius of $10R_*$ at $T \simeq 600$ K, whereas the outer optically thin component has a radius of 60 R_* at $T \simeq 375$ K. Recent work[7] replaces this two-component model by a continuous temperature distribution, but the temperatures at the corresponding radii are about the same. Much further out, the upper limit on the HCN molecular envelope[8] is 1,200 R_* and the extended CO envelope[9,10] is seen out to at least 3,800 R_*. Since Solomon *et al.*[11] observed microwave CO emission, 15 further molecules have been identified in its extended envelope, and abundance estimates are available for 14 of these (Table 1). We have performed molecular equilibrium calculations in an attempt to match the observations. We wish to determine if the relative abundances can be explained by a 'freeze-out' model: this supposes that chemical equilibrium holds close to the central star's photosphere, but that as the temperature and density fall in the circumstellar wind, molecular reaction rates drop rapidly enough for molecular abundances to be 'frozen' near their equilibrium values at some point near the star.

A suitable criterion for 'freeze-out' to apply is $\tau_{\mathrm{chem}} \gg \tau_{\mathrm{exp}}$ where τ_{chem} is the time scale for an e-fold change in the abundance of some molecule and τ_{exp} is an expansion time scale given by the time to cross a density scale-height in the shell. For IRC+10216, $v \approx$ constant[1] and we assume $\rho \propto (r/r_{\mathrm{inner}})^{-2}$, that is, steady flow. Then $\tau_{\mathrm{exp}} \approx r_{\mathrm{inner}}/v_{\mathrm{exp}} \approx 10^8$ s if we assume[12] that $r \approx r_{\mathrm{inner}} \approx r_{\mathrm{star}} \approx 10^{14}$ cm. In the outer shell $\tau_{\mathrm{exp}} \propto r$ and so the expansion time scale is longer. Calculation of τ_{chem} requires knowledge of the key reactions producing and destroying the molecules of interest and is beyond the scope of this article. However, we note that: (1) photochemical and ion-molecule reactions are probably unimportant in this neutral dusty shell (Wannier and Linke[13] report severe upper limits on molecular ion abundances); (2) if all neutral bimolecular reactions of type $A + BC \rightarrow AB + C$ had the typical rate constant $K = 10^{-11}$ cm^3 s^{-1}, then $\tau_{\mathrm{chem}}(A) = (Kn(BC))^{-1} \approx 10^4$ s to 10^8 s for $n_{\mathrm{total}} = 10^{10}$ cm^{-3} and depending on whether the reactant BC had the abundance of CO or of a less abundant species such as CH respectively. This suggests that there will be equilibrium near the photosphere ($n_{\mathrm{total}} \approx 10^{15}$ cm^{-3}; see ref. 14) and that freezing-out will start to occur for $n_{\mathrm{total}} \lesssim 10^{10}$ cm^{-3}, which is about the density at grain formation in the models of Lucy[12]. In the outer shell $\tau_{\mathrm{chem}}/\tau_{\mathrm{exp}} \propto r$ and so chemical reactions will become progressively less important. Since τ_{exp} is quite short compared with the time scales for chemical reactions in interstellar clouds[15], no chemistry at all may be going on in the outer shell, and so a freeze-out model seems appropriate.

The abundances in Table 1 have been derived both from IR absorption and microwave emission lines. Because of the radio telescope beam widths involved (about 60″), we must justify the merging of these two abundance sets. For HCN and CO, Hall and Ridgway[16] derive column densities N(HCN) > 1.5×10^{18} cm^{-2}, N(CO) = 10^{20} cm^{-2} from IR absorption lines and so N(HCN)/N(CO) > 1/67; this agrees satisfactorily with the ratio N(HCN)/N(CO) = 1/60 derived by Kwan and Hill[17] from their model and microwave observations, and is not very different from the ratio 1/150 found by Morris *et al.*[18]. However, Allen and Knapp[19] have recently derived N(CO) = 4×10^{17} cm^{-2} from the $J = 2 \rightarrow 1$ ^{13}CO line, in substantial agreement with the earlier figure of 2×10^{17} cm^{-2} found by Wilson *et al.*[8]. These emission measurements are averages over a beam of diameter about 1′. Wannier *et al.*[20] report a strong radial abundance gradient for CO in IRC+10216. If the gradient is steep enough, then a large beam will effectively dilute the observed line strengths roughly in proportion to the area of the beam. The absorption measures are with a beam whose area is about 400 times smaller and so we might expect them to lead to a column density about 400 times larger, or about 10^{20} cm^{-2}, as found by Hall and Ridgway[16]. Thus the absorption and emission measurements for CO seem to be roughly consistent. There may be difficulties if the density gradient is less steep than about $1/r^3$. We have adopted the absorption measurements in Table 1. The beam dilution effect has been allowed for by Morris[1], who used a $1/r^2$ density model in deriving column densities for SiS, SiO, CS and HC_3N, and so his emission measurements are on essentially the same scale as the absorption measurements. The value adopted for CN comes directly from the emission measurement[19] of the ratio to CO; for C_2H we derived a column density from the data in ref. 21. For C_4H and C_3N we do not know in detail how the column densities were

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Table 1 Molecules observed in IRC + 10216 and comparison with calculated equilibrium abundances

Molecule	Column density (cm^{-2}) Emission	Refs	Absorption	Refs	Adopted relative abundance	Expansion velocity ($km\ s^{-1}$)	$\log\left(\frac{\text{observed abundance}}{\text{equilibrium abundance}}\right)$
CO	2×10^{17}	8	5×10^{19}	9	1	15–16	0 (by definition)
	4×10^{17}	19	10^{20}*	16			
CS	1.8×10^{15}*	1	—		1.8×10^{-5}	13	+0.6
	$CO/CS\approx10^4$	18, 42					
CN	10^{15}	19					
	$CN/CO=2.8\times10^{-3}$*	19	—		2.8×10^{-3}	14	+6.5
	$CN/CS\approx8$	18, 42					
C_3N	$10^{14}\rightarrow8\times10^{14}$*	22, 43	—		2.5×10^{-6}	13	+0.3
HCN	$CO/HCN\approx150$	18					
	$CO/HCN\approx60$	17	$\geq1.5\times10^{18}$*	16	0.015	—	−0.3
	$HCN/CN\approx3$	18, 42					
HC_3N	1.8×10^{15}*	1	—		1.8×10^{-5}	13	−0.3
HC_5N	4×10^{14}	44	—		—	—	—
HC_7N	2×10^{14}	44	—		—	—	—
C_2H	$3\times10^{14}\rightarrow2\times10^{15}$*	21	—		10^{-5}	15	+0.2
C_2H_2	—		$\geq3\times10^{19}$*	16, 45	0.3	—	−0.1
SiO	4.1×10^{14}*	1	—		4.1×10^{-6}	10	−3.3
SiS	1.6×10^{15}*	1	—		1.6×10^{-5}	13	−2.9
CH_3CN	Detection only	43	—		—	—	—
CH_4	—		2.5×10^{17}*	16	2.5×10^{-3}	—	−0.8
C_4H	$4\times10^{14}\rightarrow3\times10^{15}$*	22	—		1.2×10^{-5}	—	−0.7
NH_3	—		10^{16}–10^{17}*		$10^{-5.5}$	14	+4.0

The column densities have been measured at millimetre wavelengths in emission (radio beamwidth typically 1′) and at micrometre wavelengths in absorption (IR beamwidth typically 3″). The figure for NH_3 is from Betz (personal communication and ref. 46). The adopted relative abundances are with respect to $CO = 10^{20}\ cm^{-2}$ and are taken from the entries marked with an asterisk—see text. The presence of SiC in the form of grains has been inferred from an 11 μm emission feature[27]. The equilibrium abundances correspond to $\log P = 2$, $\log T = 3.1$.

derived; the source is described as point-like[22] and so the large-beam column densities give lower limits to the true values. For comparison with our calculations we have adopted the set of relative abundances in Table 1; we assume that they form a consistent set to within one order of magnitude.

Calculations

Chemical equilibrium calculations have been carried out for 126 molecules containing 25 elements using a program written by Clegg (described in ref. 23). The calculations cover a range of temperatures and pressures expected to occur in carbon star atmospheres, namely

$$-5 \leq \log P \leq +3, \quad 3.10 \leq \log T \leq 3.45$$

with grid spacings of 1 in $\log P$ and 0.05 in $\log T$. Two C/O ratios were used: (1) 1.76, the averaged carbon star value used by Lucy[12] and (2) 5, the value used in calculations by Tsuji[14]. The O/H ratio was chosen to agree with the CO/H_2 ratio found by Kwan and Hill[17] (assuming essentially all the oxygen is locked up in CO). All other elements were given solar abundances. Many of the molecules considered have dissociation energies uncertain by 0.3 eV, and so our calculations are only accurate to one order of magnitude. Molecular data were taken from Tsuji[14,24], updated from the JANAF tables[25]. Two situations were considered: First with no grains present, and second with graphite grains present wherever the partial pressure P_C of monatomic carbon exceeds the vapour pressure P_{V_1} of monatomic carbon over graphite; the constraint $P_C = P_{V_1}$ was imposed in those regions of the pressure–temperature plane where the supersaturation ratio $S(C) \equiv P_C/P_{V_1} > 1$ in the no-grain calculations. The results are shown in Fig. 1, in which agreement with observation is best where the density of dots is highest, in Table 1 and in Fig. 2.

Discussion

The best agreement within the range of temperature and pressure considered clearly occurs for $\log T = 3.1$, $\log P = 2$, with $C/O = 1.76$ and no grains present; Table 1 shows that the agreement in that case is remarkably good, considering the coarseness of the (P, T, C/O) grid. This suggests that most molecules are formed in equilibrium, and that the anomalies are due to special circumstances. This result is similar to that found by Hall and Ridgway[16]; however, they considered only three molecules relative to CO and compared them with Tsuji's calculations[14] with $C/O = 5$, a value for which we found much less satisfactory agreement between our calculations and observations. We confirm their conclusion that the agreement is poor if we allow graphite grains to form.

The inclusion of graphite grains by using the constraint $P_C = P_{V_1}$ gives much poorer agreement with observation. This is particularly striking for HCN and C_2H_2; in the absence of grains, reasonable agreement with observation is obtained over a wide range of P, T; when grains are added the region of agreement shrinks drastically—for C_2H_2, no P, T combination gives agreement even within an order of magnitude. This is true for $C/O = 1.76$ or 5.

This result is curious, since the supersaturation ratio is so large ($S = 32$ at $\log P = 2$, $\log T = 3.1$) that it would be very surprising if graphite grains did not form—the critical value of the ratio is usually considered to be between 2(ionic nucleation) and 10 (heterogeneous nucleation). Also the visual extinction and IR emission clearly imply the presence of grains. A clue is afforded by the two molecules, SiS and SiO, that stand out in our equilibrium calculations as underabundant: the observed abundances are down by factors of 10^3–10^4 on the equilibrium abundances. This strongly suggests that silicon is mainly locked up in SiC (or silicate) grains in IRC + 10216, as in other carbon stars[26]. An 11 μm feature is attributed[27] to SiC particles, though the absence of a 10-μm emission feature seems to rule out a significant amount of silicate grains. Morris *et al.*[28] discuss the low abundance of SiO in IRC + 10216 in similar terms. This suggests that, contrary to the traditional view[29], SiC grains form before graphite grains. There is some evidence from recent observations[26] that graphite forms further out than expected. Our calculations show that, for $\log P \approx 2$, SiC condenses out before C if $C/O \lesssim 1.1$, but we were not able to obtain good agreement with observed abundances with this low C/O ratio: species containing two or more carbon atoms are much too low in abundance. At higher C/O ratios, the condensation temperatures of C and SiC are sensitive to the pressure but never differ by more than about 200 K. Although $S(C) = 1$ at higher temperatures than $S(SiC) = 1$, we find that $S(SiC)$ rises much more sharply than $S(C)$ as the temperature is lowered. Given that grain formation is a highly non-equilibrium process it is quite possible that SiC grains deplete Si, and that freeze-out occurs, before carbon grains form. This conclusion seems to be strengthened when account is taken of the temperature differences between the grains and the radiation[30] and further work is in progress at Sussex on this aspect.

Two other molecules, NH_3 and CN, stand out as overabundant compared with equilibrium calculations, which may suggest that the freeze-out assumption is not a good one. To obtain agreement for NH_3 we would need a lower temperature and/or higher pressure while for CN we would need a higher temperature and/or lower pressure; CN is very sensitive to temperature. It is possible that both are formed on grain surfaces[31]; estimates by Turner and Gammon[32] for interstellar CN give CN/CO ratios in the right range for agreement with the observed value in IRC + 10216.

We did not include HC_5N and HC_7N in our calculations as no thermochemical data are available. However, Clegg (unpublished) found their abundances consistent with equilibrium predictions (within rather large errors) using estimated bond energies for these molecules. The abundances of some other large molecules including C_2H_4, C_2H_6, and C_6H_6 were estimated from the atomic number densities. We found that their abundances were not great enough to affect the overall calculations.

At the values of pressure and temperature that give good agreement with observation, the gas pressure is considerably higher than found in time-independent models of expanding atmospheres of cool stars. We have obtained a copy of the program used by Lucy[12] for carbon stars and computed a wide variety of models using different values of T_* (photospheric temperature), L (luminosity), M_* (mass), S_c (critical supersaturation ratio) and C/O. In all cases, the general structure of the model is similar. Grain formation always occurs at around $\log T = 3.25$, $\log P = -3$. No point in the atmosphere is anywhere near the conditions $\log T = 3.1$, $\log P = +2$, as can be seen from Fig. 1. The same applies for the model of Menietti and Fix[33] for cool stars of more normal carbon abundance, so this result is probably generally true for cool expanding atmospheres.

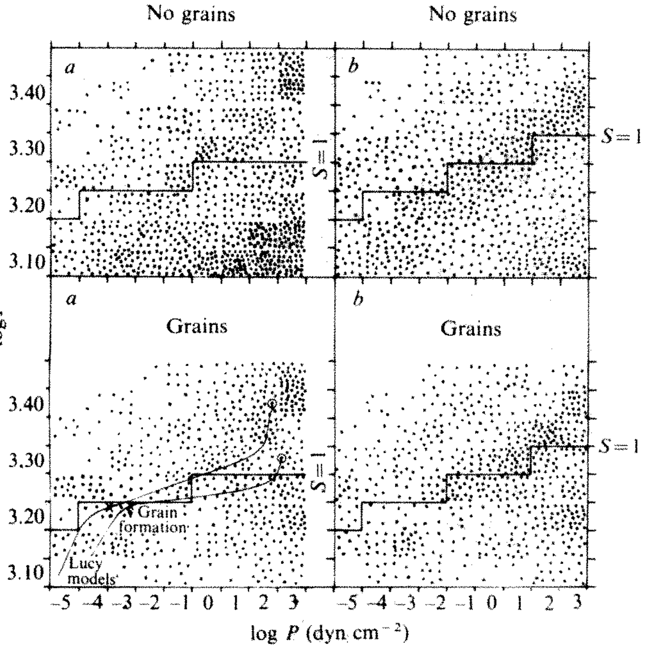

Fig. 1 A comparison of equilibrium abundances at different pressures, temperatures and C/O ratios with abundances observed in IRC + 10216. *a*, C/O = 1.76; *b*, C/O = 5. The calculations were done with grid spacings of 1 in log *P* and 0.05 in log *T*. Each grid point is represented on the diagram by a box and the results of the calculations are represented by dots within the box. Two dots indicate agreement for one molecule to within a factor of 10; six dots indicate agreement for one molecule to within a factor of 3. Lucy models for two different photospheric temperatures ($\log T_* = 3.40$ and 3.30) are shown. The grains and no-grains cases are identical for temperatures above $S = 1$ (S is the supersaturation ratio).

However, our study of the Lucy model does show (Fig. 1) that at points before grain formation we can still obtain moderate agreement with observation for some molecules, notably HCN, HC_3N, C_2H_2, CN, SiS and SiO, although for the first three this is mainly because of the wide range of P and T for which agreement is possible. Other molecules, CH_4, CS, C_4H and C_3N, disagree by several orders of magnitude. In particular, the observed abundances of CH_4 and NH_3 are factors of 10^5 and 10^7 respectively higher than they would be in equilibrium in the Lucy model before grain formation; agreement with observation can only be obtained at lower temperatures. Formation of CH_4 is known to occur catalytically on grain surfaces at low temperatures[34] and it is therefore possible that most molecules are formed in equilibrium before grain formation and then have their abundances 'frozen-in' as the reaction rates drop with density but that CH_4 and the other saturated-bond molecule NH_3 are formed far out on grains and so do not have their equilibrium abundances. However, that would not explain the other discrepant molecules.

Furthermore, there does not seem to be a unique freeze-out point in the Lucy models—there is no single P, T point giving a best agreement with observation. Radial abundance curves were plotted for all the molecules, using the Lucy model, and it was found that some molecules agree with observation before grain formation, some after and some not at all. To explain this, it is necessary to modify the 'freeze-out' picture. Equilibrium will be a good approximation only if the reaction rates are fast enough to keep up with the changes in P, T as the atmosphere moves out. Since reaction rates are proportional to number density, we expect equilibrium to fail first for the least abundant molecules. We have made rough estimates that suggest that for at least some molecules equilibrium fails before grain formation. Ion–molecule reactions could maintain equilibrium further out, but seem unlikely to be occurring in IRC + 10216, as mentioned earlier[13]. Once one molecule is out of equilibrium, all the other abundances will change and we should really do the equilibrium calculations holding fixed the abundances of those molecules that have already 'frozen out'. It would be desirable to perform a full kinetic calculation to study this problem further.

Another difficulty with the Lucy model is that the terminal velocity of the wind is considerably higher (typically 40 km s^{-1}) than observed in IRC + 10216 (see Table 1). The terminal velocity v_∞ is insensitive to most parameters of the model. But a reduction in C/O to ~1 would reduce v_∞ sufficiently and would cut down the grain density—this could explain why we find better agreement in the absence of grains, though, as mentioned earlier, preliminary calculations seem to exclude C/O $\lesssim$ 1.1. Alternatively, as suggested by Lucy[12] on other grounds, the grains in IRC + 10216 may be larger than he assumed. The reduced grain opacity could then account for a lower v_∞. SiC grains would also have a lower opacity in this temperature range[35].

An interesting test of the freeze-out model is provided by the ratio HCN/HNC. In chemical equilibrium this ratio is independent of gas pressure and abundances and only depends on the relative binding energies and the temperature (the partition functions are almost identical). The binding energy of HNC is lower than that of HCN by[36] 0.63 eV, from which we predict HCN/HNC $\approx$ 300 at $\log T \approx 3.1$. The observed ratio of antenna temperatures is 10 (Zuckerman, personal communication) but the HCN line is optically thick. The correct ratio can be obtained from $T_A(H^{13}CN)/T_A(HN^{12}C)$ and scaling up by $^{12}C/^{13}C \approx 40$ (ref. 13). Since $T_A(H^{13}CN) \approx 1.3$ K (ref. 37) and $T_A(HN^{12}C) \approx 0.4$ K (Zuckerman, personal communication) we find HCN/HNC $\approx$ 130, in fair agreement with our prediction.

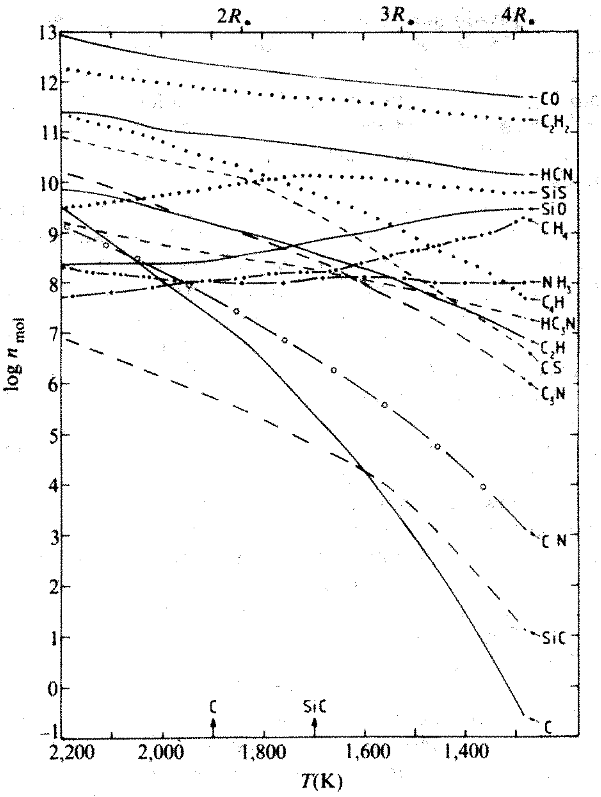

Fig. 2 Radial molecular abundance curves for chemical equilibrium based on the temperature distribution of Crabtree and Martin[7]. C/O = 1.76 and $T_* = 2{,}200$ K are specified and a $1/r^2$ density distribution is assumed. The temperatures at which S(C) = 1 and S(SiC) = 1 are indicated. No grain formation is allowed for.

Certainly the observed ratio is quite different from those found in many interstellar clouds[38], where HCN ≈ HNC.

We have found that agreement of equilibrium calculations with observation seems to be possible only in conditions of slightly lower temperature and much higher pressure than are together consistent with conventional models of steady outflow. We have therefore sought a model in which cool compressed regions arise naturally. IRC + 10216 is known to be variable on a time scale of about 600 days[39] and grains and gas may well be ejected only at one phase in the cycle and so be lost in shells or 'puffs'. We speculate that this can be invoked to form the compressed regions suggested by the high pressure found for the best fit. If an inner shell absorbs radiation from the star, it shields any outer shell. The inner shell could therefore overtake the outer shell and so perhaps compress the gas between them. This gas would also be shielded from the stellar radiation and so could cool, perhaps reaching the cool, high pressure conditions suggested by the observed molecular abundances. Close to the star there may well be a hot region, either a chromosphere or a heated shock region[40], and the dust shells would act to block UV radiation arising there. Dusty regions near such a UV source could be both a site for CN and NH_3 formation on grains[31,32] and a sink for SiS, SiO molecules onto grains. This model predicts that molecular lines found close to the stellar surface (for example, NH_3; Betz, personal communication) may be variable with time.

Whether or not this model is correct, there is no doubt that the observed abundances cannot be explained in terms of freeze-out in a conventional model of the expanding atmosphere of a carbon star. The fact that we have found a good fit for two-thirds of the molecules and can suggest why the other four molecules do not fit may mean that freeze-out does occur but that actual expanding atmospheres have much higher pressures for a given temperature than does the Lucy model. That would be the case if a $1/r^2$ density dependence were valid even close to the stellar photosphere.

Future work

Our attempt to see if the 'freeze-out' model can explain the IRC + 10216 abundances has suggested some useful future lines of investigation. First, a chemical kinetics calculation for these species in a neutral, adiabatic gas flow is desirable and is underway at Texas. Even using approximate rate constants may help to identify key reactions for the destruction and formation of the observed molecules and to evaluate the accuracy of the assumption of a unique freeze-out point for all species. Second it would be useful to construct Lucy-type models of expanding atmospheres with SiC grains rather than C grains; it is hoped to do this at Sussex in the near future. On the observational side, it is important to know the radial abundance gradients of the molecules so that we can accurately match the observations[20]. It would also be useful to have consistent measurements for all molecules, using as far as possible the same techniques and methods of reduction. Discovery of more molecules may help elucidate the chemistry in the inner shell close to the star. Finally, it would be valuable to obtain more measurements in other similar objects, such as CIT 6[19,41], to see whether the abundance pattern is repeated.

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Mélange in the Trondheim Nappe suggests a new tectonic model for central Norwegian Caledonides

Gregory S. Horne

Wesleyan University, Middletown, Connecticut 06457

Extensive mélange exists near the base of the Trondheim Nappe, indicating tectonisation of syndepositionally deformed chaotic deposits. The mélange is associated with other features that are characteristic of forearc accretionary prisms. Such a tectonic setting for the Trondheim Nappe has profound implications for the evolution of the Scandinavian Caledonides.

THE Trondheim Nappe underlies a physiographic basin south of Trondheimsfjord in the central Norwegian Caledonides[1]. It is the crestal plate of a pile of generally west-dipping nappes that crop out consecutively eastwards across Scandinavia[2]. These nappes wedge out and converge to the west and seem to represent a telescoping of Lower Palaeozoic facies from eugeoclinal rocks at the top of the pile in the west to miogeoclinal-paralic strata in lower sheets to the east[3]. Thin slices of Precambrian basement are incorporated within and between the various allochthons.

Tectono-stratigraphic setting

The Trondheim Nappe contains a complex internal stratigraphy that has experienced several phases of penetrative deformation, at least one phase of regional metamorphism, and a complex history of thrusting. Scarcity of fossils within these strata makes correlations among the various units tenuous and speculative. This has caused confusion and controversy over stratigraphic nomenclature and structural interpretation.

The succession along the eastern margin of the Trondheim region is generally of upper greenschist–lower amphibolite facies[4–6]. Lithic similarity and stratal continuity among eastern areas indicate correlation with Wolff's[4] key sequence (Table 1). Fossils are known only from an early Silurian graptolite locality in the Kjølhaug Group[4], and a Dictyonema locality in uncertain relations with correlatives of the Fundsjø Group[5]. Elsewhere, strata presumed correlative with the middle of the sequence include a significant serpentinite-pebble conglomerate with early–middle Ordovician gastropods that indicate both American and European affinities[5].

The western succession of low greenschist facies rocks in the Trondheim region reveals extreme complexity among various sedimentary and volcanic facies (P. Ryan and D. Skevington, personal communications). The nomenclature of Vogt[7] persists for major units (Table 1), but stratigraphic details are subject to progressive revision with the discovery of new faunas. Most graptolite and shelly faunas indicate Ordovician provincialism with strong North American identity[8,9]. The most significant aspect of the western sequence is the several kilometres-thick accumulation of spillitic basalts and pyroclastic strata that constitute the Støren Group[10].

The western succession is separated everywhere from the eastern succession by a belt of unfossiliferous, higher metamorphic grade schists and gneisses assigned to the Gula Group[4]. Correlations between eastern and western successions are not only governed by the stratigraphic significance of the Gula but also depend on regional structural interpretations as well as the particular tectonic model that is favoured. For example: (1) Roberts *et al.*[11] have interpreted the Gula as the older core of a mushroom-shaped antiform, correlating the eastern and western sequences as outward younging inverted limbs of that antiform. (2) Gale and Roberts[10] modified the mushroom model by lumping all the groups within the Trondheim Supergroup, conjectured to include various continental-margin and oceanic facies foreshortened by obduction before antiformal folding. Thus, the Gula was called the miogeoclinal equivalent of the eugeoclinal–backarc assemblage of the Hovin–Horg and Sulåmo–Slågån groups, also equivalent with the sea floor volcanics in the Støren and Fundsjø groups (assumed to be the same). (3) In contrast, Gee and Zachrisson[12] contended that the superficially similar eastern and western successions are quite distinct and within separate nappes. The western sequence was presumed to have been deposited on the Gula, and then both thrust over the eastern sequence, which was later thrust eastwards as the Köli Nappe.

More recent interpretations are variations of the double nappe hypothesis described above[1,2,13], but also include extensive later translation of the Trondheim sequences/nappes farther eastward across the Baltoscandic Shield.

Table 1 Major Stratigraphic units of the Trondheim Supergroup

Area	Group	Lithology	Age
Western[7]	Horg	Calcareous sandstone, shale, quartzite conglomerate	
	U. Hovin	Tuffaceous sandstone, slate, polymict conglomerate, limestone	Caradoc–Ashgill
	L. Hovin	Graywacke, slate, felsic extrusives, andesite porphyry	Arenig–Llanvirn
	Støren	Greenstone, pillow basalt, metatuff, pyroclastic deposits	
Central	Gula	Phyllite, metagraywacke, amphibolite, biotite–sillimanite schist	?
Eastern[4]	Slågån	Phyllite, slate, metasiltstone	
	Kjølhaug	Phyllite, metagraywacke, slate	Llandovery
	Sulåmo	Metagraywacke, phyllite, slivers of greenstone	? Mid-Ordovician
	Fundsjø	Greenstone, amphibolite, quartz keratophyre	? Tremadoc

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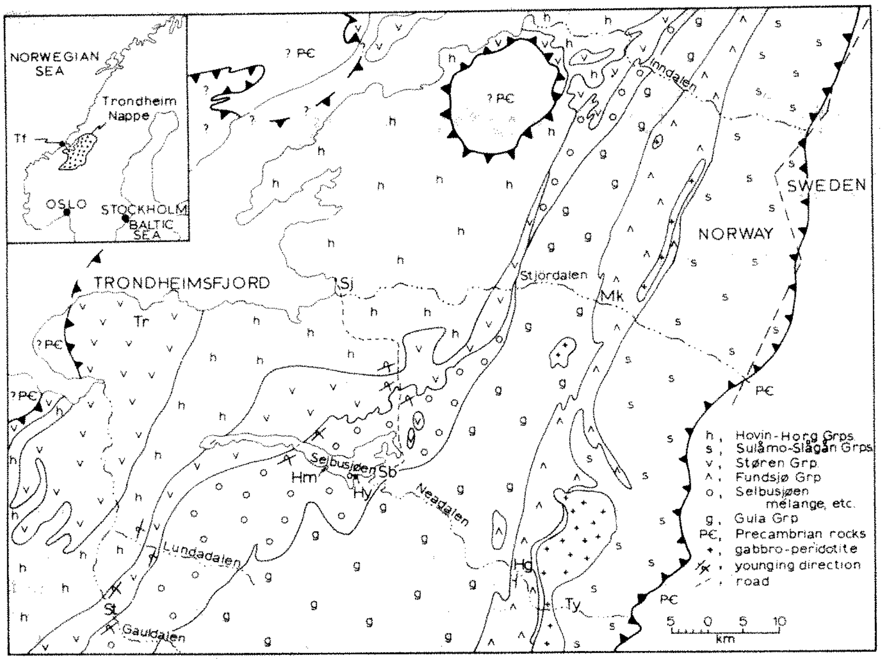

Fig. 1 Generalised geologic map of the Trondheim region, Norway (modified after Wolff[15] and Gee[2]); Hg, Hegset; Hm, Hammar; Hy, Hoöya; Mk, Meråker; Sb, Selbu; Sj, Stjördal; St, Støren; Tf, Trondheimsfjord; Tr, Trondheim; Ty, Tydal.

The resolution of these conflicting interpretations involves understanding the nature of the relationship between the Gula and adjacent volcanic groups, Støren to the west and Fundsjø to the east. The contacts have been variously described as normal sedimentary, conjunctive–tectonic, disjunctive–tectonic, and highly tectonised with thick phyllonites and mylonites. The best exposures that reveal the Støren–Gula relationship are along the shores of Selbusjøen (Fig. 1).

The Selbusjøen mélange

The succession north of Selbusjøen was divided into upper sedimentary (Hovin), middle volcanic (Støren), and lower sedimentary units[14]. The lower sedimentary unit is upper greenschist facies and is best exposed along the lake's north shore; it comprises phyllite, metagraywacke, and quartz schist. The same units extends east of Selbusjøen[6] grading progressively into coarser and higher grade metamorphic facies typical of the Gula Group. Mélange fabric was first recognised along the south shore of Selbusjøen and subsequently found on the north shore and in other areas.

Mélange fabric

The most evident aspect of mélange fabric is phyllite with fragmental debris principally of metagraywacke. Large blocks range from rounded boulders (Fig. 2) to angular phacoids; none reveal definitive evidence on origin of fragmentation. Phacoids may be boudins or pulled-apart interbeds, and boulders may have been rounded by tectonic rotation within the pervasively sheared phyllite matrix; some or all fragments also may be clasts of sedimentary origin. Less coarse debris grades through cobbles to pebbles and granules, but without apparent sorting of fragments. Some units are rich with graywacke debris, similar to pebbly mudstone (Figs 3 and 4), but even within small outcrops clast fabric ranges widely in size and angularity. In spite of deformational overprints on fragmental fabric, fragmentation seems to have been brittle in some zones (Fig. 3) and ductile in others (Fig. 4).

Volcanic material exists as lentils within the highly sheared phyllite around Selbusjøen. Discrete packets of greenstone up to several metres in length commonly lie encased within metasedimentary strata. Along the north shore of the lake apparent layers or large lentils of greenstone occur for several kilometres east of the Støren contact. The Hammar *presqu'ile* and Hoöya island (Fig. 1) consist mostly of volcanic and associated sedimentary rocks, and they may be knockers or olistoliths within the mélange. Relationships surrounding the large volcanic masses north-east of Selbu (Fig. 1) are not clear, but an olistostrome is suggested.

Associated rocks

Associated with the mélange of bouldery–pebbly phyllite are extensive zones of very highly sheared and crenulated phyllite with thin interbeds of metagraywacke–siltstone which have been mullioned into rods plunging easterly coincident with most other linear structures. Fragmentation in these zones certainly was tectonic, but could have occurred while the strata were relatively unlithified. Other thin zones between major lithic units are intensely tectonised into phyllonites and mylonites secondarily mineralised with quartz and sulphides. These latter zones were obviously deformed well after metamorphism.

In contrast to the penetratively deformed zones described above, other zones of weakly deformed and metamorphosed sediments also are found spatially associated with mélange. The physical and geometric relationships of these sediments with either the mélange or the highly sheared and mullioned zones is not clear, but contrasts in degrees of deformation and metamorphism indicate that the sediments are younger and

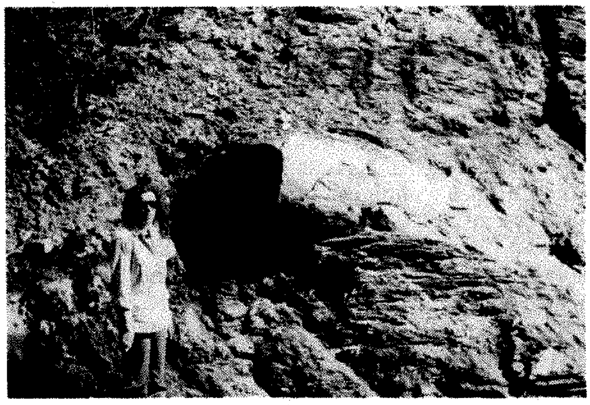

Fig. 2 Rounded metagraywacke boulder within pervasively sheared phyllite mélange, roadcut along the south shore of Selbusjøen.

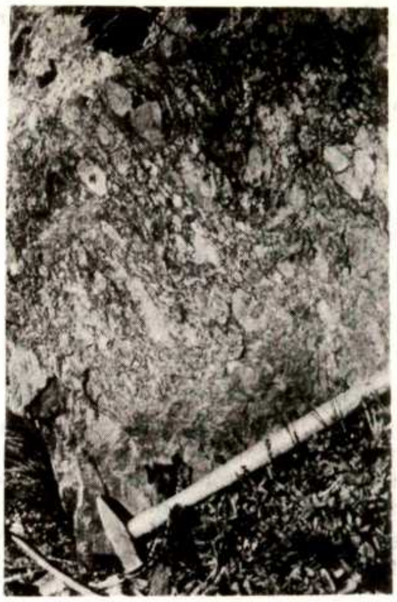

Fig. 3 Dense clast fabric of metagraywacke and volcanic debris in phyllite; note the preferred orientation of clasts, but lack of apparent flattening or stretching.

probably discordant on the more highly deformed rocks. The sedimentary strata consist of turbiditic graywacke and syndepositionally disturbed mudstone–phyllite; evidence of downslope slumping and distortion is abundant. Near the Selbu–Stjördal road weakly deformed phyllite unconformably overlies more highly deformed quartzite–phyllite–schist retrograded from almandine grade; the unconformity cross-cuts layering in the latter rocks with minor erosional relief.

Oleson and others[6] included all the rocks that crop out around the eastern part of Selbusjøen in the Gula Group. However, it is not known how these rocks relate to other parts of the Gula, or even if they should be included in that Group as it is used by others[4,5]. For instance, Wolff[15] implied that they were part of the Hovin Group. On the basis of graded bedding, truncated strata, and pillow lavas it is evident on both shores of Selbusjøen and along the Selbu–Stjördal road that the Selbusjøen rocks are overturned and lie stratigraphically beneath Støren volcanics. The contact at the base of the Støren is sharp wherever exposed and seems to be primary, but it could be tectonic. The south-eastern extent of the Selbusjøen sequence is obscured by easterly gradational and continual increase in metamorphic grade up to staurolite–kyanite schists and gneiss. The latter rocks are obviously part of the Gula Group as it is used by all workers in the region.

Regional relationship

Several major valleys (-dalen) cut through the central Gula belt and provide sections across the critical contacts with adjacent units. Gauldalen cuts through the Gula Group south-east of Støren (Fig. 1), where pillow lavas indicate that the Støren Group is overturned and youngs to the north-west. The Støren–Gula contact is clearly exposed at the south-west edge of town, and seems primary with low grade metasedimentary rocks in direct contact with greenstone; graded bedding corroborates evidence from pillows indicating the metasediments underlie the volcanics. The 'upper' (north-west) part of the Gula is similar to the rocks exposed around Selbusjøen: sheared and crenulated phyllite, graphite schist, banded metachert, weakly metamorphosed slate–graywacke, and pervasively sheared mélange with metagraywacke debris, all infested with hypadyssal sheets of trondhjemite. Geochemical studies of larger plutons of trondhjemite in the Støren area indicate derivation from low-K tholeiitic basalt by partial fusion[16]. Five kilometres south-east of Støren, rocks of higher metamorphic grade and more typical of the Gula Group are in tectonic contact with the rocks described above; cataclasites and submylonites are present within the abrupt and narrow contact zone.

The Lundadalen section lies midway between Støren and Selbusjøen (Fig. 1) and also shows Støren Group pillow basalts to be overturned and to young westward into the exposed inverted contact with the Hovin Group of sedimentary strata replete with younging criteria. The Støren–Gula contact is not exposed, but the two groups are pervaded, respectively, with quartz keratophyre and trondhjemite sheets near the contact. A narrow zone at the western side of the Gula belt adjacent to the Støren Group consists of sheared phyllite containing both volcanic and metasedimentary debris; this zone is followed eastward by alternating belts of metagraywacke, greenschist, phyllite, metachert, amphibolite, and biotite gneiss, with numerous highly tectonised shear zones.

Neadalen extends south-east of Selbusjøen to Tydal (Fig. 1) and clearly shows the gradational increase of metamorphic grade easterly through the Gula Group. Excellent exposures in the ravine at the Hegset Dam show west-dipping pillow lavas of the Fundsjø Group to young into a sharp and steeply west-dipping fault contact with the Gula Group to the west. This is one of the few exposures of the Fundsjø Group that contains unequivocal younging direction, a point of regional controversy and tectonic significance[3,4]. Of comparable significance is the presence of the 5 km-thick mafic layered igneous complex just east of the Fundsjø outcrop belt; the bowl-shaped body lies encased within an envelope of sheared pelitic rocks and it contains cumulate structures indicating that it is not inverted[6].

Stjördalen extends from Trondheimsfjord towards the Swedish border (Fig. 1); it contains the controversial section that bears on the various structural interpretations of the Trondheim region[2,4,11,12]. This section shows a fanning of structural surfaces from east-dip at the west through vertical in the centre to gentle west-dip at the east; major tectonic wedging[3] must be implicit in any model that explains the fan structure. None of the critical contacts is exposed in the Stjördalen section. The Støren–Gula contact is a 1-km-wide tectonised zone of sheared alternating slices of metavolcanic and metasedimentary rocks of various textures and metamorphic grades. The Gula–Fundsjø contact

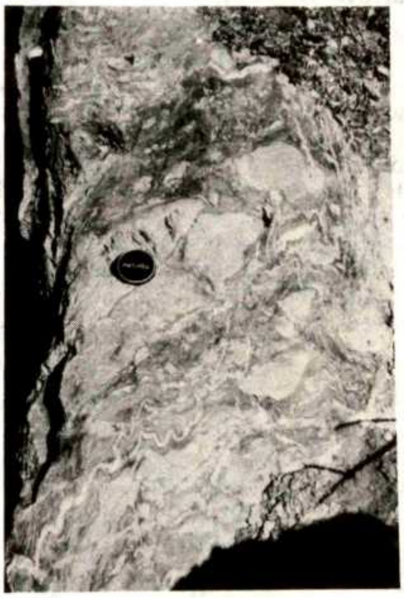

Fig. 4 Phyllitic pebbly mudstone with unsorted and unoriented fragmental debris of graywacke; note apparent disaggregation of block edges.

has been pervasively infested by massive sills of diorite and concordant sheets of trondhjemite.

The Inndalen section (Fig. 1) is similar to the Stjördalen section with its fanning structure. The contacts of the Gula Group with adjacent volcanic groups are not well exposed, but are highly sheared zones pervaded with layers of diorite, trondhjemite, and quartz keratophyre. The eastern contact of the Fundsjø Group is also tectonised and followed eastward by various metasedimentary strata and zones of phyllite mélange, which are separated by a major strike fault from another slice of metavolcanics and the succeeding Sulåmo–Slågån Groups farther to the east.

Summarising the critical relationships among the major rock units from west to east: (1) the contact between the Støren Group and the Selbusjøen mélange (and associated rocks) appears to be primary; (2) the contact between the Selbusjøen rocks and rocks more typical of the Gula Group is usually tectonic, but south-east of Selbu it seems to be gradational; (3) sheets and larger bodies of trondhjemite are commonly associated with both the Selbusjøen rocks and the Gula Group; (4) at least at one locality the Fundsjø Group demonstrably youngs to the west into a fault contact with the Gula Group; (5) mélange is also present east of the Gula Group associated with the Fundsjø–Sulåmo contact; (6) a regional fanning of structural surfaces exists from the Hovin Group in the west to the Sulåmo Group in the east.

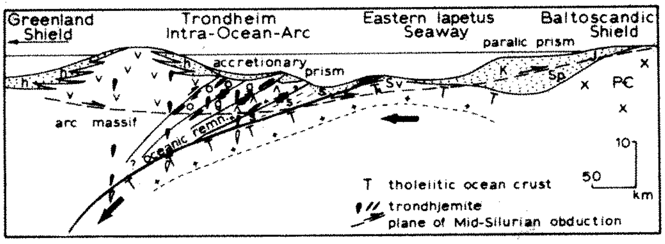

Fig. 5 Hypothetical schematic section through Early Silurian intra-ocean-arc complex within contracting Iapetus Ocean, showing relationship of various Trondheim Supergroup units to accretionary prism; J, Jämtland Supergroup; K, Köli Supergroup; Sp, Eocambrian Sparagmite; Sv, Seve Supergroup; other symbols as on Fig. 1.

The accretion model

No single criterion is exclusively indicative of any unique environment, but associations of many features are usually the best indicators of most tectono-sedimentary settings. Although mélange genesis is not restricted to forearc environs, mélanges are commmonly characteristic of that tectonic setting[17-19]. Moreover, mélanges are often associated with other features which together indicate subduction zones and the resulting accretionary prisms[19-23], such as: packets of slumped upper-slope apron deposits (as seen overlying the Selbusjøen mélange); localised internal unconformities within accretionary basins (as near the Selbu–Stördal road); localised conglemorates internally derived from submarine ridges of accreted slices (as in the Sulåmo–Slågån Groups); slices of oceanic volcanics included among the slices of accreted sediments (as within the Selbusjøen mélange and in the Stjördalen section); tectonised contacts between the various accreted slices, with development of shear zones, phyllonites and mylonites (as seen in each of the sections described here); inclusion of pods and sheets of basal ophiolite sequences or layered mafic complexes and ultramafics (as near Tydal); gross fanning structure across the accretionary prism of imbricate thrusts, from low-lying slices in the front to steep wedges at the rear (as the fan structures in the Stjördalen and Inndalen sections); anatexsis of oceanic crust leading to infusion of the accretionary prisms by swarms of hypabyssal derivatives of partial fusion of tholeiitic basalt (as in the Gula Group and Selbusjøen rocks).

Figure 5 shows how a model of a forearc accretionary prism applied to the Trondheim Nappe may explain much of the structural-stratigraphic complexity in the region. The model suggests that oceanic lithosphere from an eastern Iapetus seaway was subducted westward beneath an intra-ocean-arc system consisting of low-K tholeiites and associated sublittoral deposits, which are now represented by the western succession of the Trondheim Nappe. As the eastern seaway closed through the Ordovician, slices of outer slope and abyssal sea floor deposits, and remnant slivers truncated from culminations on oceanic crusts, were accreted into and to shallow depths beneath the leading edge of the arc system; these slices are now represented by the eastern succession of the Trondheim Nappe. The central succession of the Gula Group and Selbusjøen mélange probably represent foreshortened inner slope and trench deposits squeezed between the arc front and accreting sea floor slices, resulting in initial penetrative deformation and metamorphism. Later evolution into the Silurian merely involves the obduction of the Ordovician intra-ocean-arc complex on to the converging Baltoscandic continent with additional deformation and regional metamorphism, and attendant thrusting of continental margin and basement slices both beneath and in advance of the obducted plate. Although the eastern Scandinavian nappes are not directly the topic of this article, the continued eastward translation of this obducted pile across Scandinavia could easily result in further imbrication and subdivision of its leading edge into the Jämtland, Köli, and Seve Nappes, with or without basal Sparagmites.

This model is admittedly speculative and at variance with all previous models. For example, the polarity of subduction advocated here is opposite to that proposed in most other models[3,10]. Yet, I think that this new hypothesis greatly simplifies interpretations of the regional tectonic history, and that it may help solve many of the fundamental problems in the Trondheim region. It also may apply to other comparable parts of the Appalachian–Caledonian orogen, as in central Newfoundland[24,25].

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Experimental determination of interacting sequences in ribosomal RNA

Alex Ross* & Richard Brimacombe†
Max-Planck-Institut für Molekulare Genetik, Abteilung Wittmann, Berlin-Dahlem, FRG

An approach is described for determining secondary structure in large RNA molecules, in which families of short interacting RNA fragments are isolated and identified as such by two-dimensional gel electrophoresis. Data covering over half of the 16S ribosomal RNA from Escherichia coli *are presented, including two long-range interactions.*

ELUCIDATION of the three-dimensional organisation of ribosomal RNA is essential to an understanding of ribosome structure and function. Unfortunately, progress in this direction has been hampered by the lack of suitably direct experimental methodology for the determination of secondary and tertiary structure in large RNA molecules. The techniques which have so far been applied to the problem include modification with single-strand-specific reagents such as kethoxal[1-3], binding of oligonucleotides[4,5], and analysis of the cutting points of ribonucleases[6-8]. All these methods, although they have yielded valuable information on the location of single-stranded regions, leave the central question essentially unanswered, namely, how are the double-stranded regions of the sequence base-paired with one another? For this it has been necessary to resort to predictions (either manual or computer-aided (for example, ref. 9)) of the sequences most likely to interact, an exercise which may well lead to totally erroneous conclusions, particularly in the case of the complex ribosomal RNA molecules, where evidence is accumulating that widely separated regions of the primary sequence are involved in mutual interactions[10-13]. A secondary structure prediction of the type just mentioned was made for the 16S ribosomal RNA from *Escherichia coli*[6], but in this case the situation was even more unsatisfactory, as the model not only disagreed with much of the corresponding kethoxal data[1-3], but also was derived from a primary sequence which contained a large number of errors.

In this article, we present a direct experimental approach to the determination of interacting sequences in RNA, and describe its application to the 16S RNA from *E. coli*, taking advantage of the fact that the complete nucleotide sequence of this molecule has now been established[14,15]. The method is in principle very simple. The RNA is digested with the single-strand-specific S_1 nuclease[16], and the products are separated on a two-dimensional gel electrophoresis system similar to that used by Vigne *et al.* to identify two interacting fragments of 5S RNA[17]. The first gel dimension is run in non-denaturing conditions and the second in denaturing conditions, with the result that RNA sequences which were involved in interactions in the first dimension become dissociated and appear as pairs or families of fragments in the second dimension. The nucleotide sequences of these fragment families are then determined. The results so far have yielded information on over half of the 16S RNA molecule, and are presented in the form of a working model for the secondary structure of the regions concerned, including two long-range interactions.

Isolation and controlled digestion of the 16S RNA

In this type of experiment, it is important to minimise perturbations of the secondary structure during isolation and digestion of the RNA. For this reason we have used a particularly mild isolation procedure in which the RNA is separated from protein on a sucrose gradient containing dodecyl sulphate, sodium chloride and magnesium acetate. Figure 1 shows, using ^{3}H- and ^{14}C-labelled 30S subunits, that this procedure leads to total deproteinisation of the 16S RNA, and that the RNA sediments significantly faster than a parallel sample run on our more usual gradient system containing EDTA in place of magnesium[18], indicating a rather more compact conformation. For the digestion experiments, 16S RNA was prepared in the same way from ^{32}P-labelled 30S subunits, and was treated with S_1 nuclease at *p*H 5.5 in a buffer containing the same concentrations of sodium

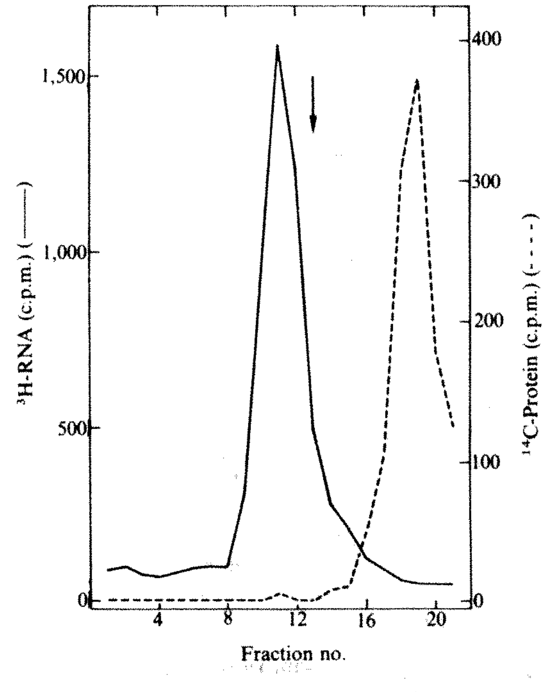

Fig. 1 Isolation of RNA. 30S subunits from *E. coli* MRE 600 were isolated as described elsewhere[25,27], and were applied to 7.5–30% sucrose gradients containing 0.1% SDS, 0.3 mM magnesium acetate, 10 mM sodium chloride and 10 mM Tris-HCl, *p*H 7.8. The gradients were run at 8 °C for 20 h at 25,000 r.p.m. The sample in this case was of 30S subunits (25 A_{260} units) labelled with ^{3}H in the RNA moiety and ^{14}C in the proteins. Direction of sedimentation is from right to left, and the arrow denotes the position of the 16S RNA peak on a parallel gradient containing 2 mM EDTA in place of NaCl and magnesium acetate. For S_1 nuclease digestion experiments, ^{32}P-labelled 30S subunits (~6 A_{260} units, 10^9 c.p.m.) were applied to the gradient, and the fractions containing ^{32}P-RNA were pooled, precipitated with ethanol and taken up in 0.1 ml of 0.3 mM magnesium acetate, 10 mM NaCl and 2 mM Tris-HCl, *p*H 7.8. The solution was brought to *p*H 5.5 by addition of 20 mM triethanolamine acetate, *p*H 5.5, and was incubated for 1 h at 20 °C with S_1 nuclease (100 units per A_{260} unit RNA), followed by a further hour with proteinase K (60 μg).

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* Present address: Biophysics Department, Portsmouth Polytechnic, Portsmouth, UK.

† To whom correspondence should be addressed.

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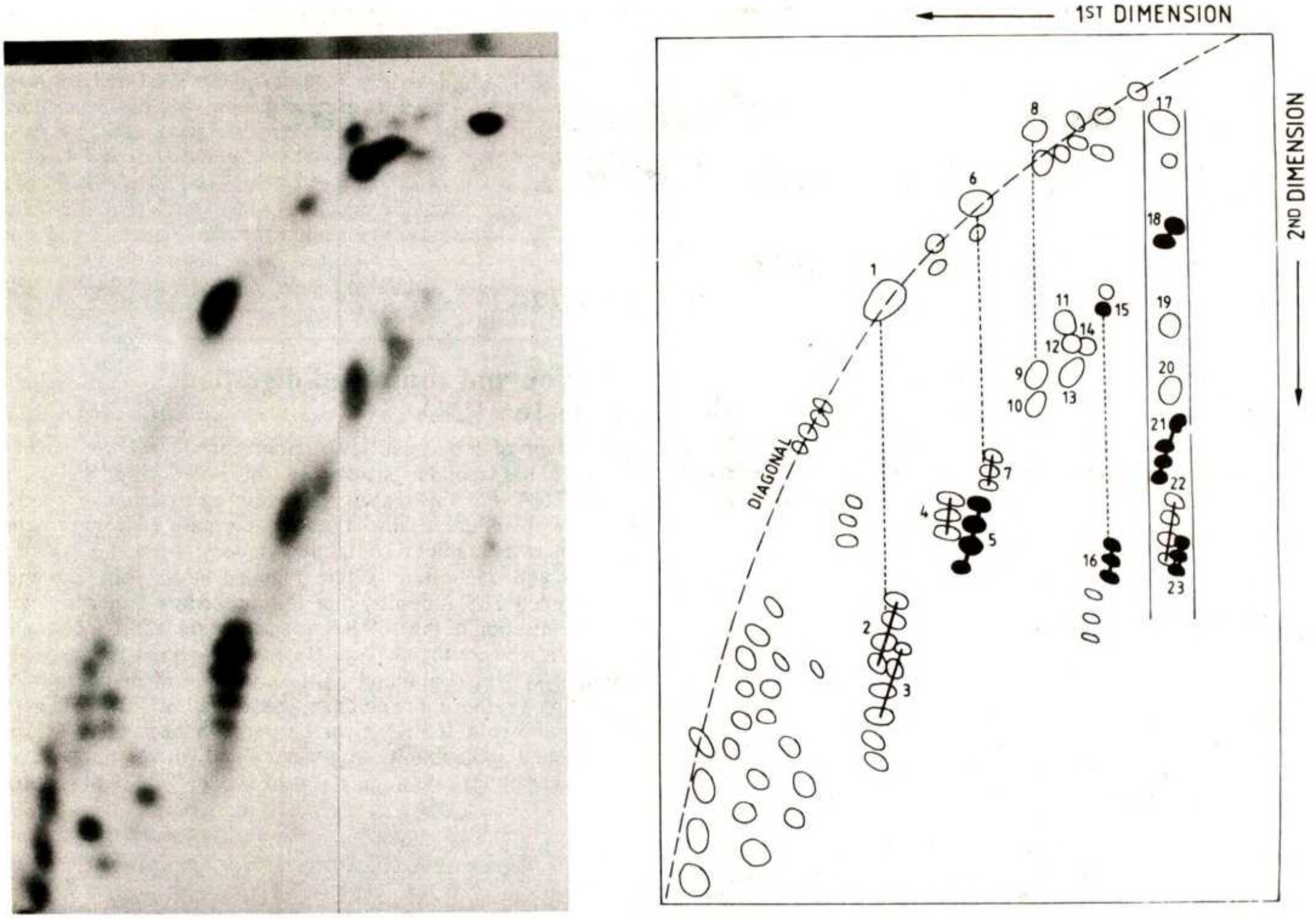

Fig. 2 Two-dimensional separation of EDTA fragments. After proteinase treatment, the S_1-digested RNA (see Fig. 1 legend) was made 6.5 mM in EDTA and then divided into two aliquots which were incubated for 10 min at either 35 or 40 °C, respectively, followed by rapid cooling in ice. The samples were applied to a 5–20% polyacrylamide gradient slab gel (40 cm long), using the dodecyl sulphate–EDTA buffer system described in ref. 13. The gel was run at 0 °C, until a bromphenol blue marker had run 24 cm. The sample strips were autoradiographed and then applied to 20% gels containing urea, using the buffer system of ref. 28. These gels were run at room temperature, until a xylene cyanol marker had run 24 cm. Two gels (15 cm × 40 cm) were needed for each sample strip, and the figure shows the combined autoradiographs of the two gels from the 40 °C sample, with the corresponding autoradiogram from the first dimension superimposed at the top. (A substantial amount of the RNA ran as mononucleotides, not included here.) The key on the right-hand side indicates the numbers assigned to the spots or groups of spots. Groups of spots joined by bars indicate families of identical sequence, deduced from the oligonucleotide analyses (for example, the upper two spots from the fragment group 2 plus 3 contained only the fragment 2 sequence, the lower two spots only the fragment 3 sequence; the poorly resolved spots in the centre contained a mixture of both sequences 2 and 3). Spots shaded in black were either only observed or were much stronger on the gel from the 35 °C sample. Vertical lines indicate sets of related fragments.

and magnesium as were present in the sucrose gradient. Details are given in Fig. 1 legend.

Preliminary tests showed that when the digested RNA was applied to the two-dimensional gel system outlined above, the number of inter-fragment interactions was far too large to permit an analysis; in the first (non-denaturing) dimension most of the RNA ran as multi-component complexes containing many hundreds of bases. It was therefore necessary to introduce a partial denaturing treatment before applying the sample to the gel. This served to simplify the pattern of fragments by destroying most of the weaker interactions (which probably also included some isolation artefacts), leaving only the most stable structures. For this purpose, the digested RNA was first treated with proteinase K to inactivate the nuclease, and was then denatured by warming to specific temperatures in various buffers. After rapid cooling, the sample was applied to the two-dimensional gel system. (Subsequent experiments revealed that the proteinase treatment did not totally destroy the enzyme in our conditions. This fact was advantageous, in that it allowed a further mild digestion of some of the single-stranded fragments released during the denaturing incubation, resulting in a further simplification of the gel patterns; on the other hand, this same effect may be partly responsible for the ragged ends observed in the fragments (see below).)

The gel patterns obtained were very reproducible for a given denaturation treatment, but varied markedly according to the precise conditions used for this denaturation. We will describe the results of two sets of experiments, one in which the sample was denatured in the same magnesium-containing buffer as that used for the nuclease incubation, and the other in which the magnesium was removed by addition of EDTA. The sets of fragments will be referred to as 'magnesium' and 'EDTA' fragments, respectively.

Fragments partially denatured in the presence of EDTA

Figure 2 shows the two-dimensional gel pattern obtained from an S_1 digest of 16S RNA, partially denatured in the presence of EDTA. Precise conditions for the sample preparation and gel electrophoresis are given in the legend. Denaturation was made at either 35 or 40 °C, and the key diagram on the right of Fig. 2 indicates the additional spots which were observed at the lower temperature, as well as those which were present at both temperatures. The figure shows the expected properties, namely, a 'diagonal' consisting of fragments either already denatured or containing uncut loops of secondary structure, together with clear families of fragments lying below this diagonal. (The diagonal is curved as a result of the gradient gel system used for the first dimension.)

^{32}P-labelled RNA fragments were extracted from the gels, digested with T_1 ribonuclease and subjected to oligonucleotide analysis by the classical techniques of Sanger *et al.*[19]. The gel separation was sufficiently good and the background sufficiently low for clean fingerprint data to be obtained in most cases without further purification, and a typical fingerprint (bases 577–615 in the 16S sequence[14]) is shown in Fig. 3. Molarities of the oligonucleotides present were determined from the fingerprint, and their identities were confirmed by digestion with ribonuclease A as described in Fig. 3 legend. The data were then fitted to the 16S sequence of Brosius *et al.*[14], and the fragment analyses from the gels corresponding to both denaturation temperatures (35 and 40 °C) were combined. From these analyses, a picture of the gel pattern in terms of nucleotide sequences could be built up, fragments of identical sequence (but differing in steps of one nucleotide in length) being indicated by the bars joining the spots in Fig. 2. The location in 16S RNA of the sequence corresponding to each numbered spot or group of spots from Fig. 2 is presented in Fig. 5. It is clear from the groups of related spots in Fig. 2 that in many cases the fragments had 'ragged ends', and in addition it was not always possible to localise the 5′ and 3′ ends of the fragments precisely, either as a result of the particular region of the 16S sequence concerned (such as a fragment ending in a run of several G residues), or due to an ambiguity in the identification of the 5′ and 3′ oligonucleotides (which are of the type pXpYpZp and XpYpZ, respectively, after consecutive S_1 and T_1 digestion). Thus, the 5′ and 3′ ends of the fragments as depicted in Fig. 5 should be taken as being approximate to the extent of ±2 bases. In all cases, however, there was excellent agreement between the fingerprint data and the length of the fragment as estimated by its mobility in the second-dimension gel.

Comparison of the data of Figs 2 and 5 shows that the expected fragment relationships appear. For example, fragment 1 (occurring about 600 nucleotides from the 5′ end of 16S RNA) is the sum of fragments 2 and 3, but the latter are not quite contiguous sequences, being separated by a gap of several nucleotides. Further up the gel, a similar situation is found with the larger fragments 6 and 7, from the same region of the RNA, except that in this case (in contrast to spots 2 and 3) the spots containing fragment 7 were a mixture of both halves (denoted 7 and 7′ in Fig. 5) of the sequence contained in fragment 6, again with a gap in the middle. Thus, fragments 2–3 and 7–7′ must be involved in base-paired interactions with one another, and run in the first dimension together with the corresponding uncut loop fragments 1 and 6. The overall pattern of fragments from Fig. 2 will be discussed below, together with the results from the set of magnesium fragments.

Fragments partially denatured in the presence of magnesium

Figure 4 shows the two-dimensional gel pattern obtained after denaturation at 45 or 50 °C in the presence of magnesium. A different gel system was used for the first dimension in this case (see Fig. 4 legend) to keep magnesium present as far as the second dimension, and the acrylamide concentration was also increased to improve the resolution of the smaller fragments. In other respects, Fig. 4 should be interpreted in exactly the same way as Fig. 2. The pattern of fragments is obviously rather more complex in this case, and in some parts of the gel (such as the spots between nos 28 and 29) the fingerprints obtained were mixtures which could not be clearly interpreted. Again, gels corresponding to both denaturation temperatures were analysed, but in this case, for simplicity, the spots on or near the diagonal (with the exception of nos 42 and 48) were not subjected to fingerprinting. It is interesting that RNA prepared by the standard phenol–dodecyl sulphate technique or by acetic acid–urea treatment[20] gave identical gel patterns to that of Fig. 2 after digestion followed by denaturation in EDTA. However, when the digest was denatured in the presence of magnesium, RNA prepared by either of these methods gave patterns resembling that of Fig. 2 more closely than that of Fig. 4.

The results of all the analyses from the gel of Fig. 4 are included in Fig. 5, and the structures which were deduced for the interacting fragments are presented in Fig. 6. Figures 2, 4, 5 and 6 should be considered together in the following discussion, fragments 1–23 arising from the gel of Fig. 2, and 24–50 from that of Fig. 4.

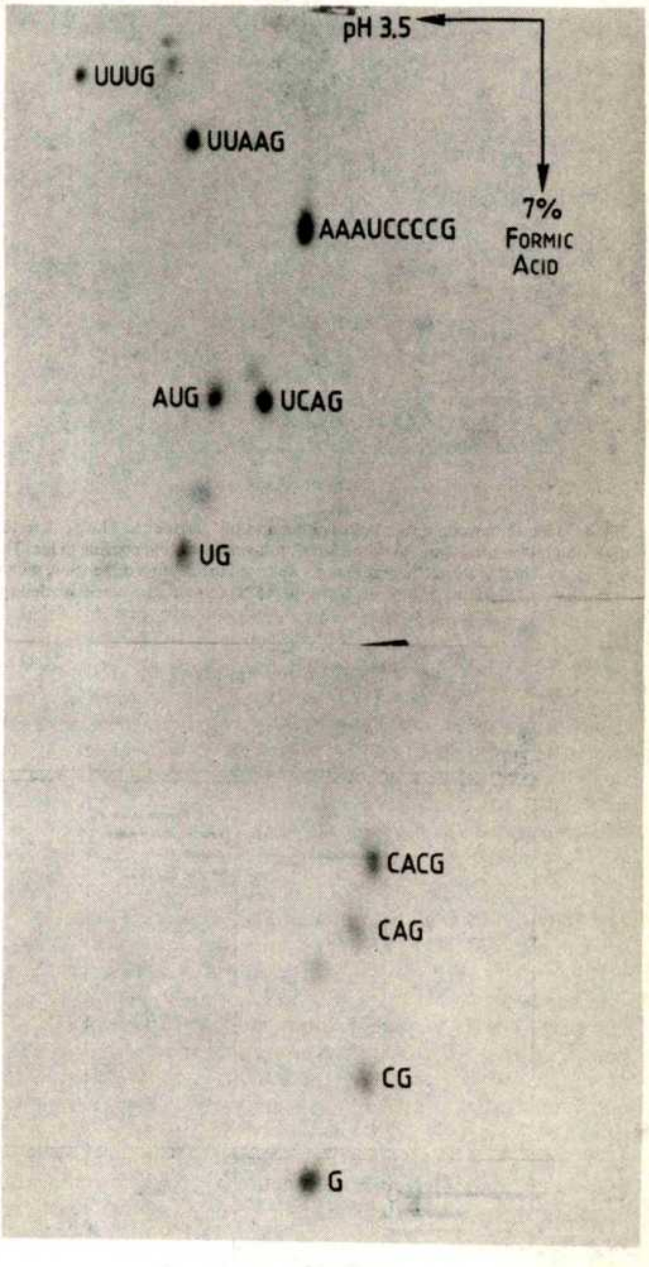

Fig. 3 Example of a T_1 ribonuclease fingerprint. ^{32}P-labelled spots were extracted from the two-dimensional gels (Figs 2 or 4) with phenol and dodecyl sulphate buffer as described elsewhere[25]. Each RNA sample was precipitated from the aqueous layer with ethanol, in the presence of 50 μg carrier RNA, and, after a further ethanol precipitation, was dissolved in 10 μl of 10 mM Tris-HCl, *p*H 7.8, and 1 mM EDTA, and digested with T_1 ribonuclease. Oligonucleotide analysis was made according to the methods of Sanger *et al.*[19] as described elsewhere[25]. The spots were cut from the fingerprints, analysed for radioactivity and subjected to secondary digestion with ribonuclease A as described elsewhere[29], using the two-dimensional separation system of ref. 30. The fingerprint in this case is of bases 577–615 of the 16S RNA sequence[14].

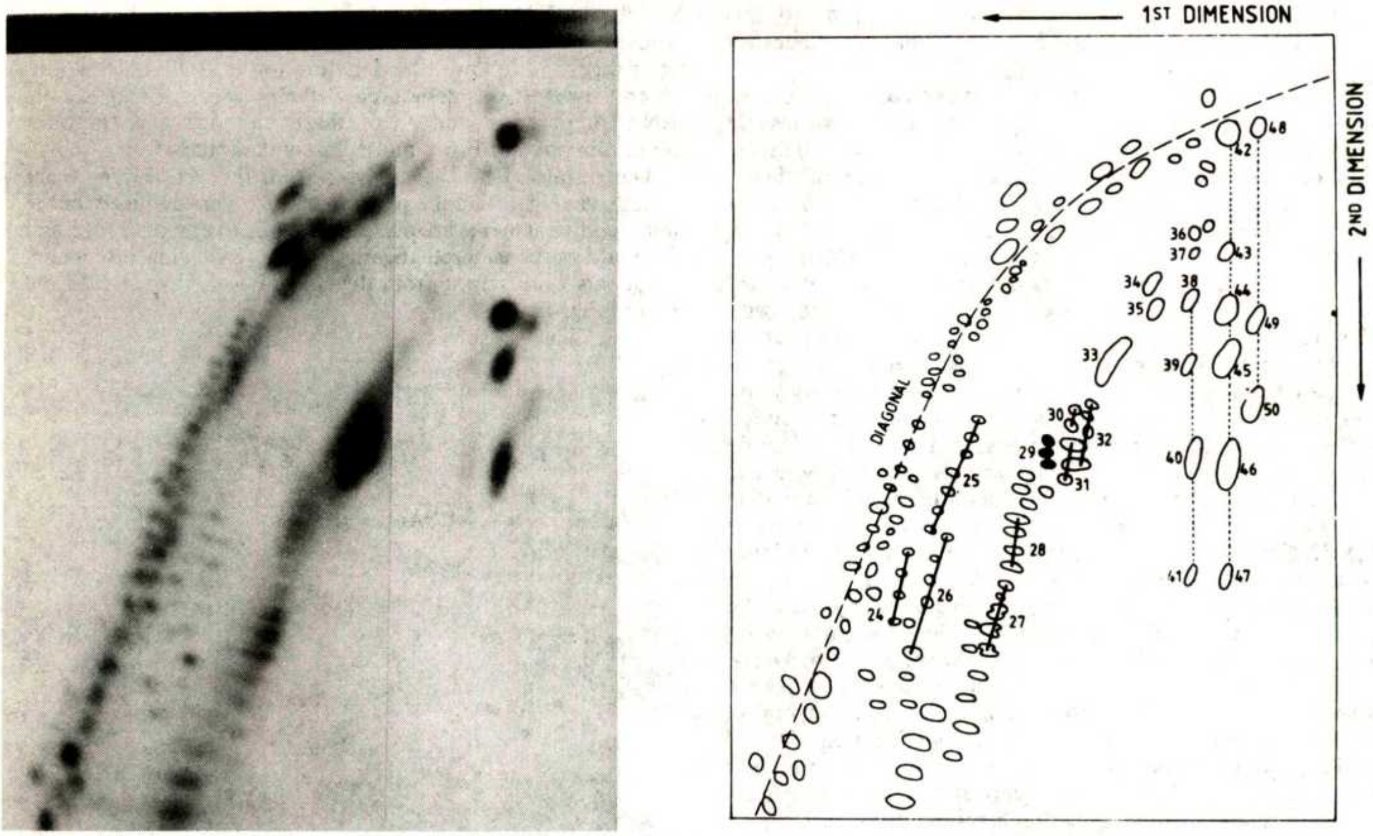

Fig. 4 Two-dimensional separation of magnesium fragments. The S_1-digested RNA (see Fig. 1 legend) was divided into two aliquots which were incubated in the digestion buffer for 10 min at 45 or 50 °C, followed by rapid cooling in ice. The samples were then applied to the two-dimensional gel system as described in Fig. 2 legend, except that a 5–25% gradient gel was used for the first dimension, with 0.3 mM magnesium acetate in place of dodecyl sulphate and EDTA. The figure shows the autoradiographs of the gels from the 45 °C sample, the black-shaded spots (no. 29) being absent in the 50 °C sample. For other details see Fig. 2 legend.

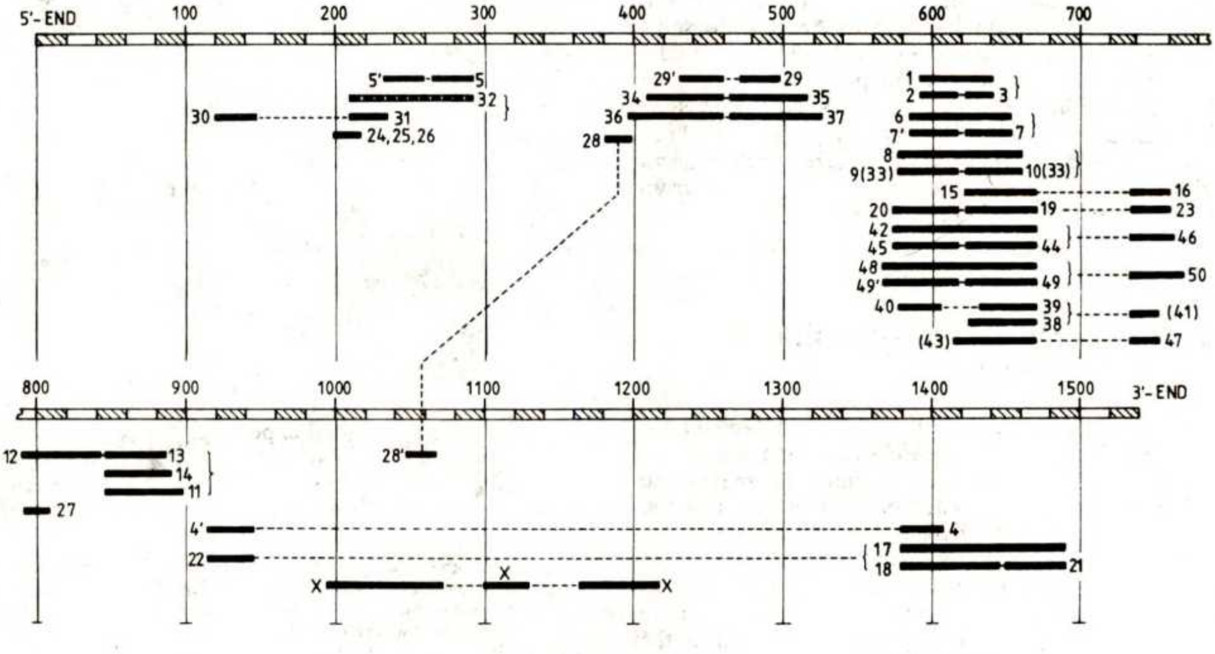

Fig. 5 Location of fragments in the 162 RNA sequence[14]. A scale of nucleotides is given, numbered from the 5' end. Fragment numbers by each sequence (black bars) correspond to those of Figs 2 and 4, and interacting fragments are joined by dotted lines. Sequences such as 29, 29' were found together in the single gel spot with the corresponding number. Fragments bracketed together (for example, 1 and 2, 3) were on the same vertical line in the gel, whereas fragment numbers in parentheses (for example, 33) indicate minor components. Fragment 32 is a special case which (judged from its mobility in the second dimension gel) contained at least three sequences corresponding to fragments 5, 5' and 31. The three fragments marked X were found in earlier experiments using RNA isolated in the presence of EDTA and denatured to 35 °C in the presence of 100 mM NaCl after digestion.

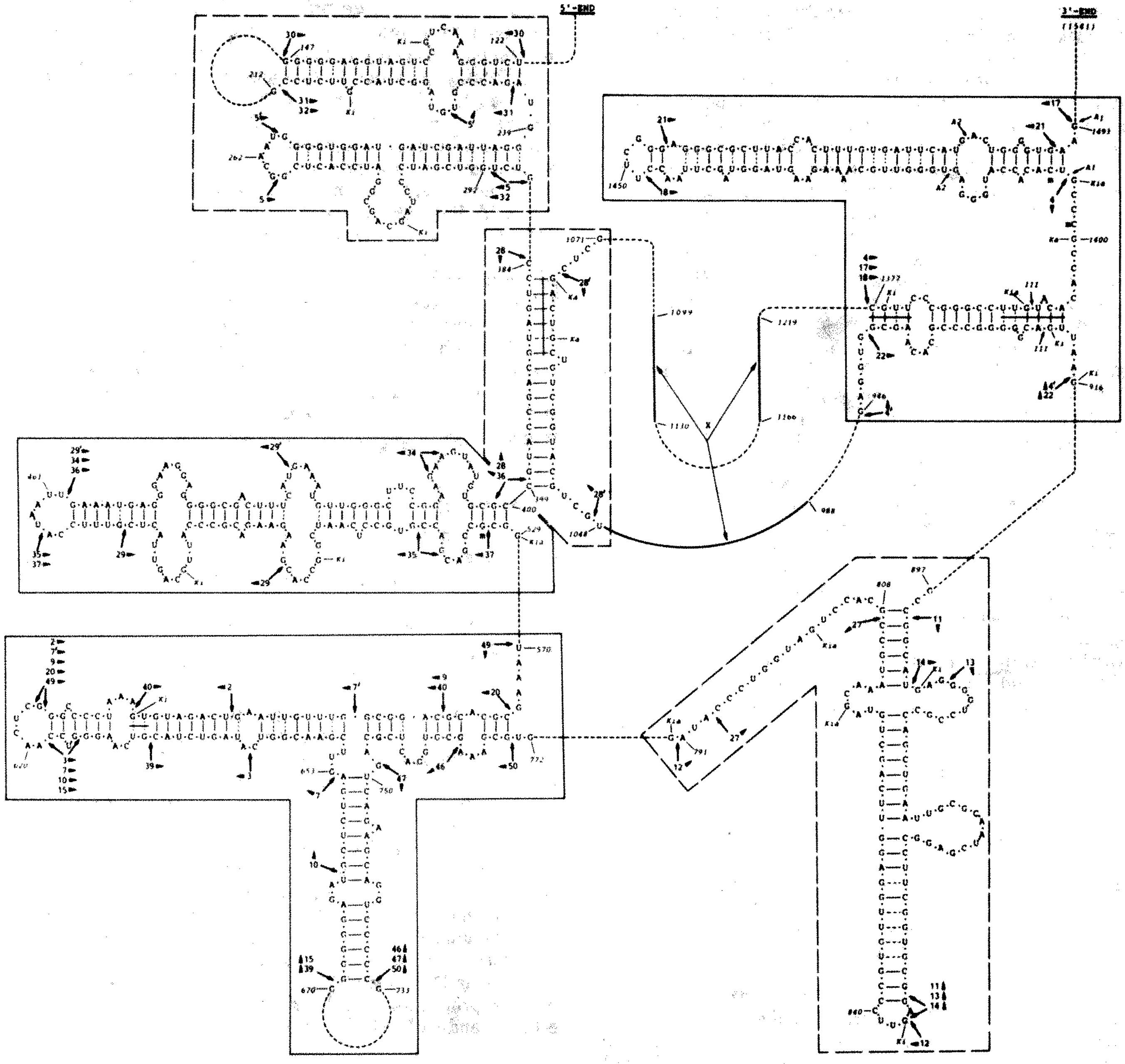

Fig. 6 Secondary structures proposed for the interacting fragments. Numbers in italics indicate the distance in nucleotides from the 5' end of the RNA. Numbers in bold type indicate the approximate 5' and 3' ends of the respective numbered fragments from Figs 2, 4 and 5, with the arrowheads indicating in which direction each fragment runs. The structural domains enclosed by solid lines are regarded as quite certain, whereas those enclosed by broken lines are less certain, for the reasons discussed in the text. Kethoxal sites are indicated by Ki and Ka for sites in inactive[3] or active[1,2] particles, respectively, and bars drawn through base-paired regions (for example, bases 385–390) are where the kethoxal data suggest a single-stranded structure in the 30S subunit. The symbols III, A1 and A2 refer to fragments isolated from the 30S subunit in earlier work[25,26] and the three fragments marked X are involved in a complex interaction (see text).

Interpretation of the sequence data from the sets of interacting fragments

The criteria for interaction between two fragments are that (1) they lie on the same vertical in the gel, (2) they are present in equimolar amounts, and (3) their combined molecular weights are consistent with their mobility as an undissociated complex in the first dimension. The interactions which were deduced are incorporated in Fig. 6 into six structural domains which are surrounded either by solid or broken lines according to the degree of certainty assignable to the various structures drawn for the interactions. Best fits were obtained with the help of a simple computer program. In several cases alternative structures can be drawn which differ in detail without greatly affecting the overall topography of the loops (compare ref. 7). We have selected the possibilities which seem the most likely, but we emphasise that the important feature of our data at this stage is that the sets of fragments provide a sound experimental basis for determining those regions of the RNA which are somehow involved in interactions with one another. Note that the data agree well with the sites of kethoxal modification of RNA in the 30S subunit[1–3]; these sites are included in Fig. 6.

The simplest case to consider is the set of fragments between bases 400 and 500 (Fig. 5), consisting of the fragment pairs 29–29', 34–35 and 36–37. This set forms a clear 'pyramid' of fragments of increasing length, which can be drawn as the rather symmetrical structure shown in Fig. 6, remembering (as already mentioned) that the 5' and 3' ends of the fragments were not always precisely definable. Fragments corresponding to the shortest base-paired regions (bases 450–460 and 465–475) were not found; such small fragments would have run on the gel below fragments 24–27 (Fig. 4), an area which was too complex to interpret. In this context, remember also that S_1 nuclease does not attack all apparently single-stranded regions in RNA (for example, in tRNA only the anticodon loop is cut[21]).

Immediately adjacent to the structure just discussed, there is a strong long-range interaction of 15 base pairs, defined by the fragment mixture 28–28′. The structure is assigned as tentative in Fig. 6 because, although the interaction was reproducibly detected and the fingerprint data were clear, the sequences concerned have not so far been observed in larger fragment structures where each component of the interaction could be observed separately. In this case, the kethoxal sites suggest that the weaker half of the structure is opened up in the 30S subunit; such a situation would be consistent with the finding that the RNA has a lower helical content in the subunits than in the isolated form[22].

The structures drawn for the interactions in the regions between bases 100–300 and 800–900 are also assigned as tentative. In the latter case, the interacting fragments 12 and 11 or 13 were reproducibly found, but the presence of fragment 14 without a 'partner' (Fig. 2) suggests that the 5′ half of this loop (fragment 12) is easily digested to smaller pieces which were not analysed in the experiment of Fig. 2. The structure proposed for this interaction has a single-stranded 'tail' at the 5′ end (again suggesting a missing piece), this arrangement being supported by the finding of precisely this tail as an unpartnered fragment (no. 27) in Fig. 4. In the region of bases 100–300, a simple loop structure could be drawn for bases 231–296, containing the interacting fragments 5 and 5′ (Fig. 2). However, the sequence of fragment 5′ overlaps that found for fragment 31 (Fig. 4) by several bases, the latter fragment clearly interacting with fragment 30. An interaction similar to that between 5 and 5′ was nevertheless also present in the gel of Fig. 4, as evidenced by spot 32, whose fingerprint indicated a mixture of the sequences contained in fragments 5, 5′ and 31. In this context, the significance of the small fragments (24, 25 and 26, Fig. 4), which overlap fragment 31 and extend it in the 5′ direction, is not clear.

The most detailed information was obtained from the region of bases 550–800. For simplicity, only selected fragments are indicated in Fig. 6, and the reader should refer to Fig. 5 for fragments of similar or identical sequence (for example, fragment 15 is the same as nos 19, 44, 49 and 38). As already mentioned, the interacting fragments 2–3 and 7–7′ clearly define the loop structure of bases 587–653. The next pair of fragments (9 and 10) can be drawn as a simple base-paired extension to this structure, but the families of larger fragments contradict this simple extension. Fragments 44, 45 and 46 (Fig. 4) reproducibly showed the presence of an extra piece of RNA (bases 733–764), which must be involved in the structure, and fragments 49, 49′ and 50 indicate a further elongation in the extreme 5′ and 3′ directions. The interaction between the three fragments is accordingly represented by the structure shown in Fig. 6, and other minor components of the gels from this RNA region are consistent with the structure. For example, fragments 39, 40 and 41 appear as a family in which both the left and right ends of the structure are missing (Fig. 6), and fragments 42 and 48 correspond to uncut loops running approximately from bases 570 to 670. Fragments 15 and 16 (Fig. 2) are also consistent, although here a piece of RNA must be 'missing' from the analysis to account for the mobility of the complex in the first gel dimension. Note also that this structure agrees exactly with the secondary structure of part of this region proposed by Müller *et al.* for the RNA binding site of proteins S8 and S15 (ref. 23).

Fragments 44, 45 and 46 (Fig. 4) were identical to fragments 19, 20 and 23 (Fig. 2), respectively. In the latter case, fragment 23 disappeared at the higher temperature (40 °C, Fig. 2), allowing the complex between 19 and 20 to run slightly faster in the first dimension. This enabled the complicated region at the right-hand side of Fig. 2 to be resolved, as it follows that fragments 19, 20 and 23 form one family, and 17, 18, 21 and 22 another (Fig. 5). These fragments suggested the long-range interaction between bases ~920–940 and 1,380–1,400, an interaction which was very precisely and reproducibly confirmed by fragments 4 and 4′ (Fig. 2). This is probably the same interaction as that proposed (but not localised) by Ehresmann *et al.*[24], although as with the other long-range interaction already discussed the distribution of kethoxal sites suggests that the weaker ends of the base-paired structure are open in the ribosome. This structure also fits well with the 5′ and 3′ ends of the RNA from ribonucleoprotein 'fragment III', isolated from the 30S subunit[25], and encompasses a complex interaction between the RNA fragments named 'X' from this region. The latter fragments were found in earlier experiments, but can be arranged together in too many different ways to merit drawing a structure at this stage. The remainder of the region (bases 1,400–1,500) can be drawn as a simple loop structure, fragment 17 (lying just below the diagonal) representing the uncut loop together with the tail interacting with fragment 22, whereas fragments 18 and 21 represent the corresponding loop halves, which disappeared at the higher temperature (Fig. 2). This structure also agrees well with the sequences of two RNA fragments (denoted A1 and A2) which were isolated from the 30S subunit[26].

Conclusion

The experiments described here offer a basis for determining the secondary structure of ribosomal RNA. Although the data were obtained from isolated RNA, in general they are in good agreement with other data from the 30S subunit, in such cases where a comparison is possible. There are, however, some anomalies, suggesting structural heterogeneities in the RNA. Such heterogeneities could reflect multiple coexisting structures *in vivo*, or may represent artefacts introduced by the isolation procedure. Experiments now in progress, using the 30S subunit as starting material as well as RNA isolated by various methods, should help to resolve this aspect of the problem. At the same time it cannot yet be entirely excluded that some conformational rearrangements occur during the partial denaturation treatment of the digests, but this should become clearer as the data accumulate. We believe that the further development and application of this approach should lead not only to a reasonably accurate experimental model of the ribosomal RNA structure both within the subunits and as an isolated moiety, but also to an understanding of the relative stabilities of the individual parts of the structure and their dependence on ionic environment.

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Two-step binding of eukaryotic ribosomes to brome mosaic virus RNA3

Paul Ahlquist, Ranjit Dasgupta, Ding S. Shih, David Zimmern & Paul Kaesberg

Biophysics Laboratory of the Graduate School, and Biochemistry Department of the College of Agricultural and Life Sciences, University of Wisconsin-Madison, Madison, Wisconsin 53706

Although brome mosaic virus RNA3 has only one translatable cistron, it can bind two 80S ribosomes at initiation. One ribosome binds at the first AUG codon (bases 92–94). The other binds nearer the 5′ end at an entry or holding site. Disome formation is thus unrelated to a silent cistron ~1,000 bases downstream.

ALTHOUGH the messenger-sense genomic RNAs of many viruses infectious to eukaryotes encode several cistrons, only the 5′ proximal cistron of each such RNA is translatable[1]. Translation in eukaryotes is generally considered to be restricted to 5′ terminal cistrons by initiation requirements for a distinctive RNA sequence and a 5′ terminal blocking group—the cap. However, sequences preceding coding regions show no obvious common features[2] and the cap itself is dispensible[3]. Removal of the cap reduces the rate but not the specificity of initiation[4], and several viral mRNAs have been found that are not capped. Moreover, eukaryotic ribosomes are capable of recognising internal cistrons on uncapped prokaryotic mRNAs[5], although it is possible that such RNAs may have been modified by enzymes existing in the cell-free extracts. The characteristics that define a functional eukaryotic ribosome binding site and prevent such a site from occurring internally in the RNA thus remain undetermined.

We have examined these issues as they apply to the brome mosaic virus (BMV) RNAs. BMV is a small, icosahedral virus of cereal plants. Much is known about the location of the BMV coding regions and their translation. The BMV genome consists of three distinct RNA molecules (designated RNA1, RNA2 and RNA3 in decreasing order of size) encapsidated in three virions[6]. These RNAs are necessary and sufficient for infectivity, but virions containing RNA3 (2,200 bases) also contain one copy of a subgenomic RNA designated RNA4 (900 bases). RNA3 contains the nucleotide sequences and genetic information of RNA4, and can act as a precursor to RNA4 found in progeny viruses resulting from infection with RNAs 1, 2 and 3 alone. *In vitro* translation experiments show that RNA4, although dispensible for infectivity, is the functional mRNA for the viral coat protein. The only translation product detectable when BMV RNA3 is used as a template in optimal conditions is a polypeptide (MW 35,000) unrelated to coat protein (MW 20,000)[7]. Thus RNA3 contains at least two cistrons—an accessible cistron and a coat protein cistron which for some reason is inactive. Subsequent experience has shown that similar genomic RNAs containing one or more untranslatable coding regions, plus their accompanying subgenomic RNAs, which are active messengers, are common in viral systems[1]. The untranslatable coding regions of these genomic RNAs are now popularly called silent cistrons.

During our experiments we observed that, in cell-free extracts of wheat embryo, two ribosomes can bind to RNA3 in conditions where initiation can proceed but elongation is inhibited. In identical conditions RNA4 binds only monosomes. Originally it was considered possible that the binding of two ribosomes was related to the dicistronic organisation of BMV RNA3. However, BMV RNAs 1 and 2 also bound two ribosomes and they, as well as RNA4, are thought to be monocistronic messengers[7]. In fact, the binding of two ribosomes in similar circumstances has been observed with several viral RNAs including tobacco mosaic virus genomic RNA[8], alfalfa mosaic virus RNA3[9], and encephalomyocarditis virus RNA (our observation). As these RNAs differ considerably in their organisation and structural features, it seemed likely that some rather subtle structural feature common to all these viral RNAs was responsible for disome formation, and that this might be important both to the reproduction of the virus and to the mechanism of protein synthesis. Accordingly, we studied ribosome binding to RNA3 using procedures designed to reveal features of the nucleotide sequence involved in disome formation.

Ribosomal binding to RNA3 and to 5′ terminal fragments of RNA3

Two ribosomes bound to one molecule of ^{32}P-labelled BMV RNA3 when translational elongation was blocked by sparsomycin[10] anisomycin[11] or T2 toxin[12] used singly or in combination (Fig. 1*a*). (T2 toxin, a derivative of 12,13-epoxytrichothecene, inhibits the formation of the first dipeptide bond but has no effect on the formation of 80S initiation complexes.) In all cases in which ribosomes were present in excess, the amount of labelled RNA present in structures sedimenting at the rate of disomes exceeded that sedimenting as monosomes by at least a factor of two. In Fig. 1*a* the smaller peak of radioactive RNA coincided with the peak of UV absorbance due to 80S ribosomes. The larger peak co-sedimented with the disome peak from polysome profiles run in parallel gradients. We observed similar patterns with BMV RNAs 1 and 2 (data not shown), but not with BMV RNA4 (Fig. 1*b*), which presented only a single peak of RNA radioactivity associated with monosomes.

Although the binding experiments above were done with uniformly labelled RNA, for ease in correlation with sequence analysis it is convenient to use terminally-labelled RNA. We chose to label BMV RNA3 at its 5′ end with the capping enzyme from vaccinia virus cores described by Moss and co-workers[14]. This enzyme transfers a GMP moiety from GTP to 5′-diphosphorylated RNA termini and then, in the presence of *S*-adenosyl methionine, methylates the resulting inverted G residue and the 5′ terminal ribose of the chain proper. When partially purified, the enzyme is associated with a 5′-triphosphate γ-phosphatase. It has the important feature that it regenerates the natural 5′ terminal configuration of capped messenger RNA. The reconstructed 5′ end does have a ribose methylation lacking in the original BMV RNA, but this has been shown to have little effect on protein synthesis[16]. The enzyme has three other advantages over polynucleotide kinase, the enzyme most frequently used for 5′ labelling RNA. First, despite the fact that decapped and dephosphorylated BMV RNA3, like some other eukaryotic mRNAs[17], is an extremely poor substrate for polynucleotide

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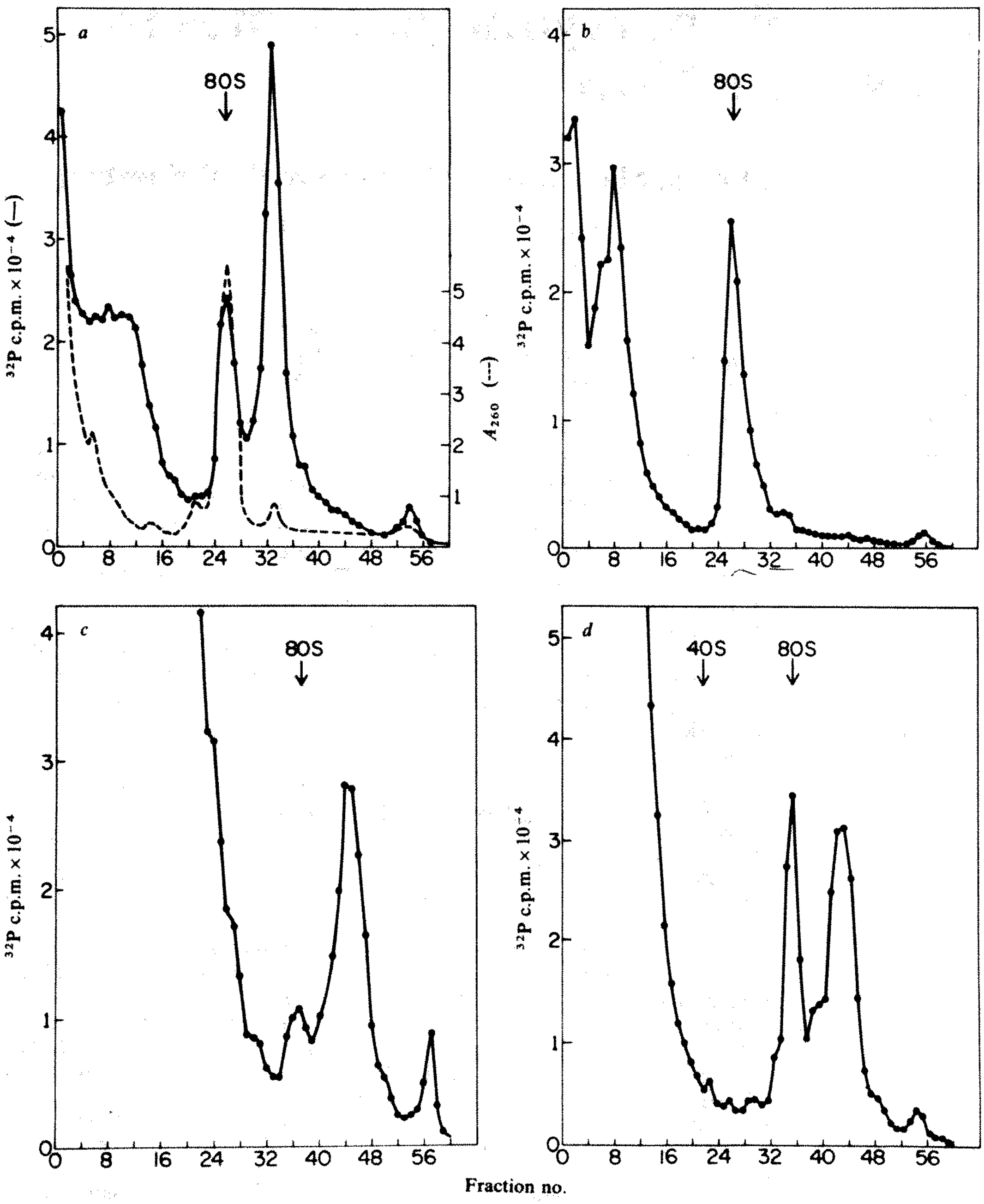


Fig. 1 Detection of the binding of two ribosomes to RNA3 by sucrose density gradient sedimentation analysis. Preparation of wheat embryo extracts and ^{32}P-uniformly labelled BMV RNA components were as previously described[13]. Terminal labelling with the vaccinia capping enzyme[14] was carried out in a 50 μl reaction mix containing 20 μg decapped BMV RNA3, 20 μM [α-^{32}P]GTP (480 Ci mmol^{-1}, Amersham), 0.16 mM ATP[15], 50 μM *S*-adenosyl methionine, 50 mM Tris-Cl *p*H 7.8, 1 mM $MgCl_2$, 1 mM DTT and 5 μl of DEAE-cellulose purified enzyme preparation. The reaction mix was incubated for 15 min at 37 °C, phenol extracted twice, ether extracted twice, and ethanol precipitated. Conditions for ribosome binding reactions (100 μl) were as described elsewhere[13] except that the creatine phosphokinase concentration was 20 μg ml^{-1} instead of 125 μg ml^{-1}. The reaction mixtures were incubated with 0.45 mM anisomycin plus 0.06 mM T2 toxin (*a* and *b*) or wiith 0.2 mM sparsomycin (*c* and *d*) for 5 min followed by addition of ^{32}P-labelled RNAs and further incubated for 10 min. The reaction mixtures were cooled on ice and applied onto 10–40% sucrose density gradients prepared 50 mM Tris-HCl, *p*H 7.6, 50 mM KCl and 4 mM Mg-acetate. The gradients were centrifuged in a Spinco SW41 rotor at 36,000 r.p.m. for 3 (*a* and *b*) or 4 h (*c* and *d*) and fractionated with an ISCO gradient fractionator. *a*, 5 μg ^{32}P-uniformly labelled RNA3; *b*, 10 μg ^{32}P-uniformly labelled RNA4; *c*, 5 μg ^{32}P-terminally labelled RNA3; *d*, 5 μg ^{32}P-terminally labelled RNA3, partially digested with RNase T_1 as described in Fig. 2, phenol and ether extracted, and ethanol precipitated before ribosome binding. The increase in ^{32}P counts at the top of the gradients shown in *c* and *d* compared to *a* and *b* is due to the presence of unincorporated [α-^{32}P]GTP from the labelling reaction.

kinase, the vaccinia enzyme consistently gave high incorporations of ^{32}P. Second, even our partially purified preparation was comparatively free of RNAse. Third, as the enzyme recognises only 5′ di- or tri-phosphorylated ends, it does not label internal nicks in the RNA. Figure 1*c* shows that BMV RNA3, decapped by periodate oxidation and β-elimination, then recapped with the vaccinia capping enzyme and [α-^{32}P]GTP in the presence of *S*-adenosyl methionine, binds to wheat embryo ribosomes in conditions of sparsomycin inhibition in a manner similar to that of uniformly labelled RNA3.

Fragments of terminally-labelled BMV RNA3, partially digested with nucleases, also bind two ribosomes in the presence of sparsomycin. Figure 1*d* shows the binding pattern of a partial RNase T_1 digest of 5′ labelled, capped and methylated RNA3 in conditions of sparsomycin inhibition. Partial RNase ϕ1 digests show a similar pattern. Preliminary experiments showed that the size of these fragments was well within the range of high-resolution sequencing gels, so in further experiments we used partial digestion conditions that gave relatively uniform cleavage of every susceptible site over the first few hundred residues and allowed us to pinpoint the parts of the RNA responsible for binding. For detailed analysis of these experiments we needed to determine the 5′ terminal sequence of RNA3.

5′ Terminal sequence of BMV RNA3

The sequence of the first 164 residues beyond the capping group $m^7G^{5'}ppp^{5'}G$ was determined by rapid gel methods using base-specific ribonucleases[18,19] as shown in Fig. 2. We determined the first few residues from the 5′ terminus independently by partial digestion with nuclease P_1 followed by mobility shift analysis using two dimensional cellulose acetate electrophoresis and homochromatography[21] to allow unambiguous counting of the bases and to confirm the sequence of the extreme 5′ end. The sequence is shown in Fig. 3.

On initial sequencing gels, pyrimidine assignments were made by examining the RNase ϕ1 cleavage pattern on each side of the base in question. Contrary to the published dinucleoside hydrolysis frequencies[22] but in agreement with other observations[23] we found that some A—C bonds (those between residues 12-13, 53-54, 99-100, 131-132, and 141-142) were resistant to RNase ϕ1 cleavage in our conditions. In later gel sequencing experiments RNase Phy M was used to distinguish between C and U residues. RNase Phy M, which cleaves after A and U residues (H. Donis-Keller, personal communication), has a much simpler base specificity than RNase ϕ1 and thus provides a more accurately interpretable cleavage pattern.

RNA fragments associated with monosomes and disomes

5′ End-labelled RNA fragments recovered from the monosome and disome regions of sucrose gradients such as that shown in Fig. 1*d* were freed of protein by phenol extraction and electrophoresed on a sequencing gel in parallel with samples of the original digests that had not been bound to ribosomes (Fig. 4, lanes *c–h*). As a second set of controls, full length RNA3 was recovered from disomes and then digested and electrophoresed

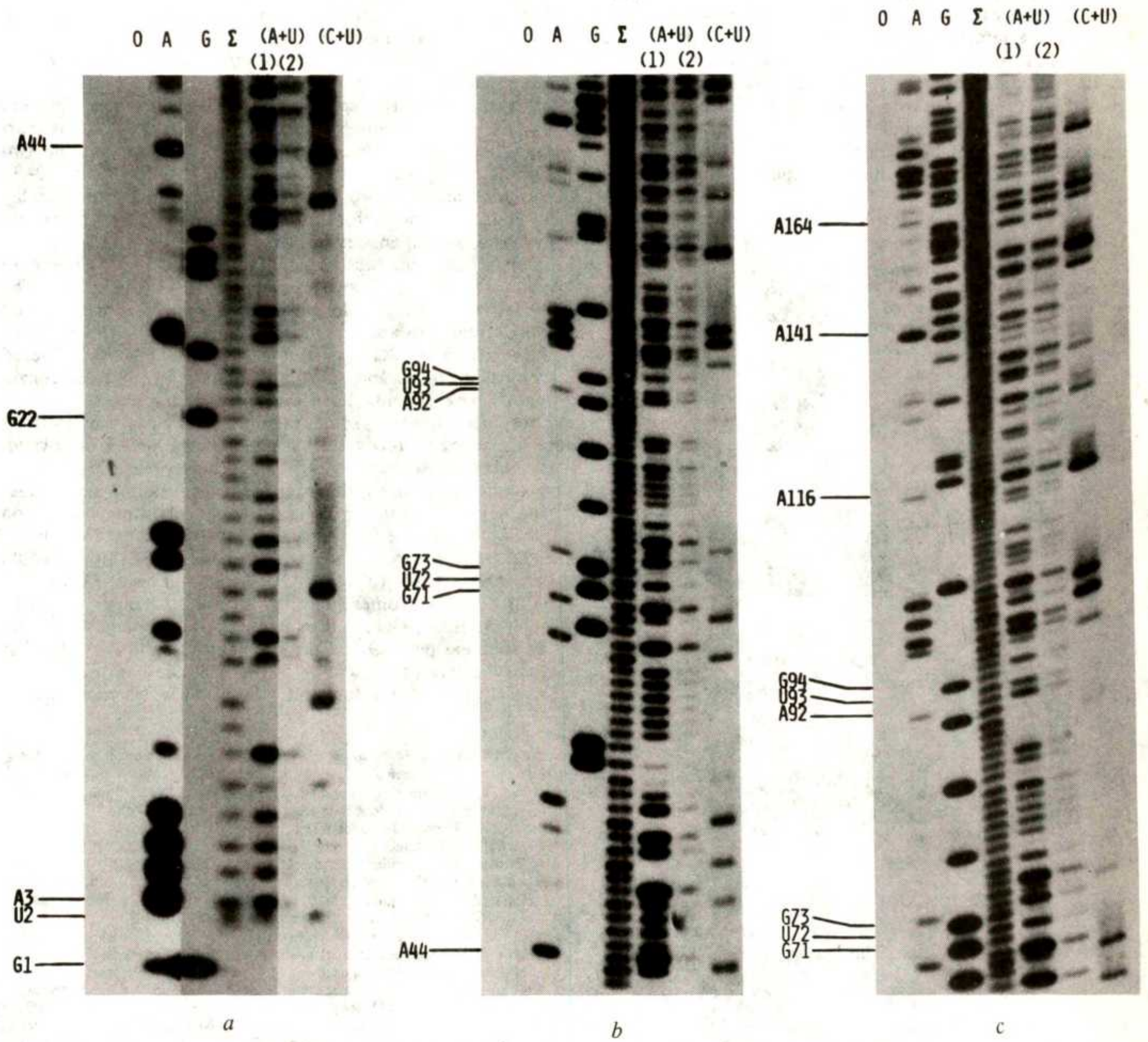
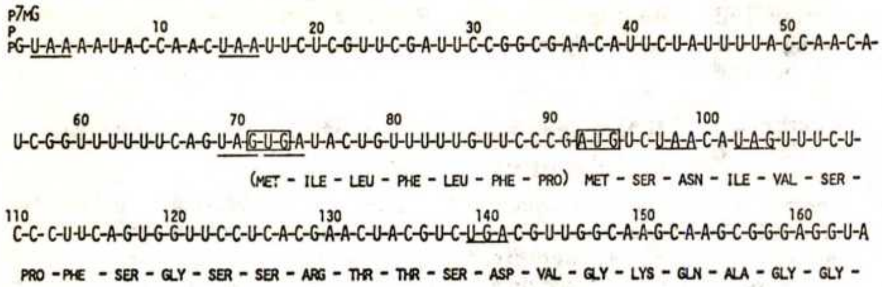


Fig. 2 Sequencing gels of 5′-labelled BMV RNA3. BMV RNA3 was 5′-labelled as described in Fig. 1, except that *S*-adenosyl methionine was omitted. Full-length labelled RNA was isolated on a 5% acrylamide 7 M urea slab gel. Enzyme digests and a minus enzyme control each contained 2.5 μg RNA in 10 μl 20 mM Na-citrate *p*H 5.0, 1 mM EDTA, 7 M urea (ultrapure urea solutions were deionised with Fisher Rexyn I-300 resin before use). Digests were incubated 15 min at 50 °C, after which 2.5 μl of 0.3% xylene cyanol, bromphenol blue, 10 mM EDTA in formamide was added to each. Two different strength digests were done for each enzyme. For RNases U2, T1, and pancreatic RNase, like enzyme digests were mixed together after incubation. For formamide digestion 5 μg of RNA in 20 μl 90% formamide, 10% double-distilled H_2O was incubated 45 min at 100 °C. An aliquot of 2 μl formamide/dye mix was then added. Samples were heated for 1 min at 100 °C and 2 μl aliquots of each were loaded onto freshly polymerised 20 × 40 × 0.035 cm 7 M urea slab gels[20] and electrophoresed at 30–35 mA in 87.5 mM Tris-borate *p*H 8.3, 2.75 mM EDTA. *a*, Shows a 12% acrylamide gel and *b* and *c* show 8% acrylamide gels. Gels were autoradiographed at −70 °C with an unflashed Kodak X-Omat R film clamped tightly between the gel and a Dupont Cronex Lightning Plus intensifying screen. The following symbols are used to designate the various lanes: O, minus enzyme control; A, 2.0, 0.2 units RNase U2 (Calbiochem) per μg RNA; G, 0.04, 0.004 units RNase T1 (Calbiochem) per μg RNA; Σ, formamide digest; A + U(1), 0.08 arbitrary units RNase Phy M per μg RNA; A + U(2), 0.008 arbitrary units RNase Phy M per μg RNA; C + U, 2×10^{-4}, 2×10^{-5} μg pancreatic RNase (Worthington) per μg RNA.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P
pG-U-A-A-A-A-U-A-C-C-A-A-C-U-A-A-U-U-C-U-C-G-U-U-C-G-A-U-U-C-C-G-G-C-G-A-A-C-A-U-U-C-U-A-U-U-U-U-A-C-C-A-A-C-A-
10
20
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40
50
U-C-G-G-U-U-U-U-U-U-C-A-G-U-A-G-U-G-A-U-A-C-U-G-U-U-U-U-U-G-U-U-C-C-C-G-A-U-G-U-C-U-A-A-C-A-U-A-G-U-U-U-C-U-
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C-C- C-U-U-C-A-G-U-G-G-U-U-C-C-U-C-A-C-G-A-A-C-U-A-C-G-U-C-U-G-A-C-G-U-U-G-G-C-A-A-G-C-A-A-G-C-G-G-G-A-G-G-U-A
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140
150
160
PRO - PHE - SER - GLY - SER - SER - ARG - THR - THR - SER - ASP - VAL - GLY - LYS - GLN - ALA - GLY - GLY -

Fig. 3 The nucleotide sequence of the 5′ terminal 164 bases of BMV RNA3. The first AUG and GUG codons from the 5′ end are boxed and nonsense codons are underlined. The amino acid sequence of the protein which would result from initiation at the first AUG is aligned with the nucleotide sequence. Extra N-terminal amino acids which would result from initiation at the first GUG are shown in parentheses.

(Fig. 4, lanes *a* and *b*). The resulting band patterns correspond exactly to those obtained by digestion of RNA3 not exposed to wheat embryo extracts (Fig. 4, lanes *e* and *h*, or Fig. 2), thus excluding the possibility that the ribosome binding behaviour we observe is an artefact of sequence rearrangement (that is, RNA splicing) in the wheat embryo extracts.

A distinct difference in length distribution was observed between RNA recovered from monosomes (Fig. 4, lanes *d* and *g*) and that recovered from disomes (Fig. 4, lanes *c* and *f*): the monosome peak contained fragments 29 to 91 residues long while the disome peak contained fragments 94 residues and longer, as well as faint traces of fragments 69 to 91 residues long (see Fig. 4 legend). The striking transition in ribosome binding properties occurring with fragments of 94 bases or longer is clearly seen on the autoradiogram as a lateral switch in the band pattern from the monosome to disome lanes.

The fact that RNA3 can bind two ribosomes efficiently, but no more than two when translation is inhibited implies that RNA3 has two sites that interact strongly with ribosomes. Efficient disome formation with 5′ terminal fragments of RNA3 as short as 94 residues and failure of shorter fragments to bind two ribosomes implies that one site is near base 94. Bases 92–94 comprise the first 5′ proximal AUG codon, which is presumably an initiation codon for protein synthesis. These bases are followed by an open reading frame as far as the known sequence extends; the other two reading frames are closed by nonsense codons following almost immediately after the AUG. The second ribosome on 94 long fragments, and thus presumably on longer fragments and on intact RNA, must be on the 5′ side of the AUG codon. As fragments 29 residues long are recovered from monosomes and shorter fragments are not, the region around base 29 must have the potential for interaction with ribosomes. However, some fragments in the range of 35 to 60 residues long are recovered from monosomes only in poor yield, possibly because the base 29 binding site may be buried by RNA secondary structure in fragments 35 residues long and longer (see below). Another potential site for ribosome interaction exists around base 70 corresponding to fragments which are again recovered from monosomes in good yield. This site would be in the vicinity of the first GUG codon at bases 71–73. The failure to form trisomes indicates, however, that on intact RNA3 stable ribosome binding does not occur simultaneously at both the site near base 29 and the site near base 70. We infer that ribosome interaction at one of the sites may be transient or sterically hindered. Consistent with this view, fragments 69–91

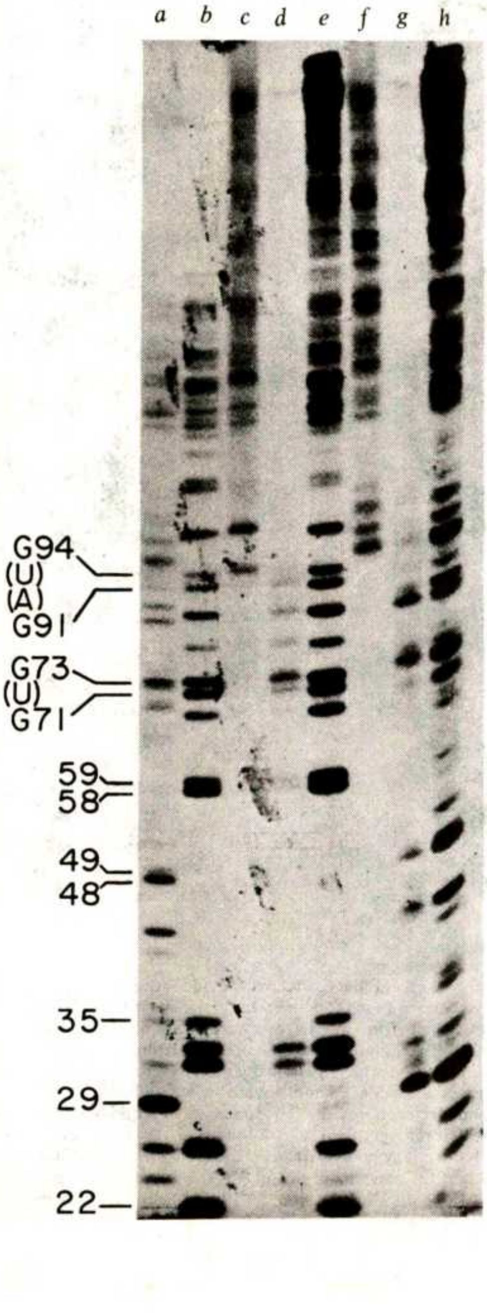

Fig. 4 Analysis of 5′ terminal BMV RNA3 fragments bound in monosomes and disomes. Terminal labelling, ribosome binding, and sucrose density gradient sedimentation were as described in Fig. 1. RNA bound to ribosomes was recovered from sucrose density gradients by pooling the desired fractions, adding 120 µg of wheat embryo ribosomes to pooled disome fractions, and ethanol precipitating the ribosome–mRNA complexes. Precipitates were suspended in 0.1 ml of 0.1 M Tris-HCl *p*H 7.6, 0.2% SDS, and extracted with 0.2 ml of water-saturated phenol. RNA was ethanol precipitated from the aqueous phase. Partial RNase digestions were performed as described in Fig. 2, except that urea was omitted from digests with RNase ϕ1, and were phenol extracted once and ether extracted twice before the RNA fragments were ethanol precipitated. The following samples of 5′ ^{32}P-labelled BMV RNA3, each in 10 µl of 8 M urea, 0.05% xylene cyanol, bromphenol blue were co-electrophoresed at 25 mA on an 8% acrylamide, 7 M urea slab gel (20×40×0.2 cm) in 87.5 mM Tris-borate *p*H 8.3, 2.75 mM EDTA: *a*, One half of the RNA3 recovered as full-length molecules from the disome peak shown in Fig. 1*c*, digested subsequently with RNase ϕ1. *b*, One half of the RNA3 recovered as full-length molecules from the disome peak shown in Fig. 1*c*, digested subsequently with RNase T1. *c*, RNAse T1 fragments recovered from the disome peak shown in Fig. 1*d*. *d*, RNase T1 fragments recovered from the monosome peak shown in Fig. 1*d*. *e*, An aliquot of the RNase T1 digest used for the gradient shown in Fig. 1*d*, saved before binding to ribosomes. *f*, RNase ϕ1 fragments recovered from the disome peak of a gradient analogous to that shown in Fig. 1*d*. *g*, RNase ϕ1 fragments recovered from the monosome peak of the gradient discussed in *f*. *h*, An aliquot of the RNase ϕ1 digest used for the gradient discussed in *f*, saved before binding to ribosomes. Long exposures of the gel shown reveal the presence of bands corresponding to fragments 69–91 residues long in lanes *c* and *f*.

bases long can form disomes, but only inefficiently, confirming that one of the two sites is poor at binding ribosomes stably.

The method above, by examining an entire set of overlapping fragments simultaneously, permits detailed analysis of the contribution of the mRNA sequence to ribosome binding. By use of suitable end-labelling techniques, this method should be generally applicable to other mRNAs, both capped and noncapped. However, it must be recognised that the nucleotide sequences of fragments may fold differently from their counterparts in intact RNA and thus their binding may be different. We have tried to evaluate the steric effect of secondary structure by systematically examining possible folding by computer. We think that it is unlikely to have affected the transition from monosome formation to disome formation between fragments 91 and 94 residues long. The onset of unfavourable secondary structure may explain however why certain 5'-terminal fragments fail to bind stably in monosomes. For example, while fragments 29, 32 and 33 residues long bind well as monosomes, the fragment 35 residues long binds poorly. The 3' end of the 35 residue long fragment can form a small base-paired stem which is at the threshold of stability (Fig. 5*a*). Fragments longer than 49 and shorter than 72 residues also bind poorly in monosomes and this may be related to stable base-pairing of the extreme 5' end of the RNA which becomes possible (Fig. 5*b*) in fragments of 50 residues or longer. Alternate base-pairing schemes of theoretical stability comparable to that shown in Fig. 5*b* can be written for the first 50 bases of BMV RNA3, and transitions between these various structures may also play a part in determining the ribosome binding efficiency of different fragments. As stable base pairing is not possible in fragments shorter than 35 or so residues, though, secondary structure effects have not influenced the transition to competence for monosome formation which occurs between fragments 26 and 29 residues long.

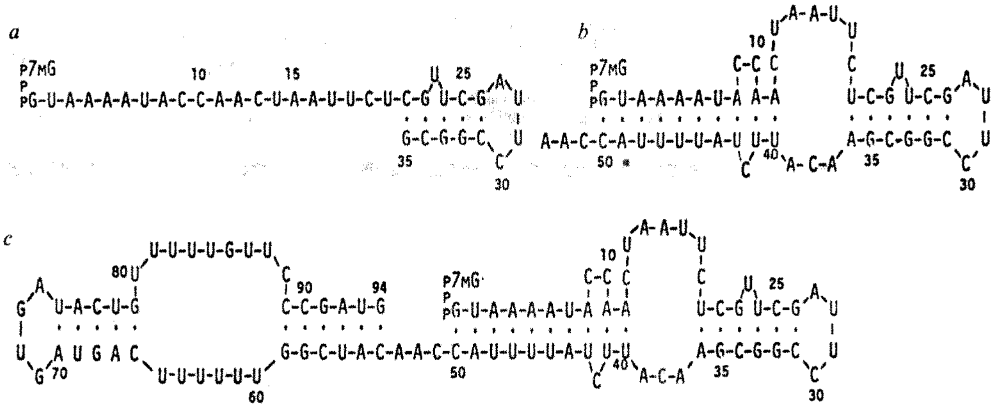

Fig. 5 Possible secondary structures for: *a*, the first 35 residues of BMV RNA3; *b*, the first 53 residues of BMV RNA3; *c*, the first 94 residues of BMV RNA3.

80S monosome formation in the absence of an AUG

Identification of the monosome peaks in Fig. 1 as complexes of mRNA with one 80S ribosome, rather than with two 40S ribosomes, is based on two observations. First, the monosome peak in each gradient coincides exactly with the peak of UV absorbance due to 80S ribosomes (Fig. 1*a*), unlike complexes of mRNA with two 40S subunits, which are believed to sediment at the 70S position[24]. Second, complexes of mRNA with single 40S subunits, which would be expected as precursors of complexes of mRNA with two 40S subunits, are completely absent (Fig. 1*d*). The report[25] that a single bound 40S subunit can protect 54 5'-terminal bases plus the cap structure of a reovirus mRNA from nuclease digestion also argues against two 40S ribosomes being able to bind the shortest RNA fragments (29 residues) recovered from the monosome peak. The monosome binding data thus make it clear that capped RNA fragments can bind wheat embryo 80S ribosomes even if they do not contain an AUG codon. 80S complex formation in the absence of an AUG codon has been described before with a capped synthetic copolymer of A and U (ref. 26), but not to our knowledge with natural mRNA 5' ends. These observations clearly conflict with the proposal of Kozak and Shatkin[27] that 60S ribosomal subunits bind to mRNA only after the 40S subunit is bound to an AUG codon. As discussed below, the possibility thus arises that initiator AUG recognition may be accomplished in normal translational initiation by the 80S ribosome rather than the 40S subunit alone.

Disome binding and two-step 80S ribosome binding

Movement of ribosomes between different sites on RNA3 is not directly tested by our experiments. However, if ribosome interaction with the site(s) 5' to the AUG has functional significance in protein synthesis it must be followed by transfer of the ribosome to the AUG site where translation commences. Disome formation with RNA3 thus indicates the existence of a ribosome entry site or alternatively a ribosome holding site. The entry site would consist of the cap plus a set of bases involved in the first stable attachment of 80S ribosomes to the RNA. The hypothetical holding site would consist of a set of bases (excluding the cap) that would accept ribosomes from an entry site and release them to a protein synthesis initiation site.

Why is there a sudden jump in efficiency of disome formation at 94 residues? Ribosome protection experiments as well as weak disome formation with fragments as short as 69 residues suggest that there is sufficient room to accomodate two ribosomes on fragments shorter than 94 residues. High efficiency disome formation therefore seems to be specifically related to recognition of the AUG codon at bases 92–94. Possibly the reason disome formation is suppressed in fragments lacking an AUG is that some feature near the 5' end of the RNA involved in binding the second ribosome remains attached to or covered by the first ribosome until the latter is stably bound at an AUG codon.

Our observations indicate that the binding of eukaryotic ribosomes to mRNA is (at least) a two step process. We suggest that eukaryotic 80S ribosomes first bind to mRNA at an entry site 5' to the initiation codon. After this binding the ribosome moves to an initiation site, recognised primarily by the presence of an initiation codon, where translation will begin. An intervening transfer to a ribosome holding site may also occur. Our observations are consistent with the possibility that 80S ribosomes move from the entry/holding site to the AUG by sliding along the mRNA, as suggested by Kozak and Shatkin for 40S ribosomal subunits[27]. Alternatively ribosomes may be brought close to the AUG by folding of the mRNA. Figure 5*c* shows a relatively weak, though stable, folding of the first 94 residues of BMV RNA3 in which the cap region and the initiation codon are adjacent to each other. After ribosome binding at the entry site, melting of the secondary structure and reorientation of the ribosome around the AUG-containing portion of the message could give a stable complex such as those revealed by nuclease protection experiments. In cases where the 5′ untranslated region of the mRNA is long enough to permit the entry and initiation sites to be physically separated, such as BMV RNA3 and TMV RNA[28], movement of the first bound 80S ribosome from the entry site to the initiation site allows a second ribosome to bind at the entry site. In other mRNAs, particularly those such as BMV RNA4 with a short 5′ untranslated region, the entry and initiation sites may overlap or coincide, allowing only a single 80S ribosome to bind when translational elongation is blocked. A fused entry and initiation site may effect a high efficiency of initiation as is the case with BMV RNA4[7], while physical separation of the entry and initiation sites might allow a more sophisticated regulation of translation.

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letters

Infrared observations of SS433

A. B. Giles & A. R. King

Astronomy Department, Leicester University, UK

B. A. Cooke, I. M. McHardy & A. Lawrence

Physics Department, Leicester University, UK

SS433 has attracted much attention following the interpretation of its optical spectrum by Margon *et al.*[1] and Liebert *et al.*[2]. A possible explanation of the Doppler-shifted Balmer emission lines has been proposed by Rees and Fabian[3] and Abell and Margon[4] in terms of two opposed jets that are ejected from the central source at speeds approaching 0.25*c* and may extend for ~50 AU (~7×10^{14} cm). Previous work has shown SS433 to be variable at radio wavelengths[5], X rays[6], IR[7,8] and also in the visible[9,10]. We report here simultaneous observations at IR and visual wavelengths which show that SS433 has an IR excess. We attribute this to a region of gas that radiates by free–free and free–bound emission processes with a total emission measure of the order of 10^{61} cm^{-3}.

We observed SS433 in bands *BVJHK* on three nights during the week 14–21 May 1979, from the Cabezon Observatory, Tenerife, using the 1.5 m IR flux collector. Our observations were made using the Leicester University IR/visual photometer with a standard set of filters and an InSb detector operated at 63 K. The standard used was θ Aql, the magnitudes being taken from Johnson[11]. The reduced fluxes are shown in Table 1 together with the phases (ϕ) computed from the ephemeris of Martin *et al.*[12].

These results show three pronounced features: first, an enormous apparent excess of IR over optical flux; second, an almost flat spectrum for wavelengths >1.2 μm; third, a variation of IR and optical flux together on a time scale of several days. The first feature is certainly exaggerated by the heavy reddening of the object and may even be entirely due to it, so we have plotted in Fig. 1 corrected fluxes, assuming a value for the interstellar extinction of $A_v=6$, and a standard extinction curve[13]. The errors on each point are too small to represent on the figure. Taking $A_v=6$ corresponds to Clark and Murdin's[14] estimated colour excess of $E(B-V)=2$.

The correction for reddening leaves clear evidence for an intrinsic IR excess; for $A_v=6$ the IR points all lie above the extrapolation of the optical continuum, whose slope also agrees well with the spectrophotometry of Margon *et al.*[1], corrected for $A_v=6$.

This conclusion is further supported by Glass' (ref. 7) observation that $K-L=0.71$. With $A_v<6$ the IR continuum is even flatter. Since it is generally assumed (see ref. 1) that $A_v\geqslant5$ we shall consider $A_v=6$ as a representative case; it will be clear how the following remarks are affected by assuming other values of A_v. In particular, for large assumed values of A_v, the optical continuum is always much steeper than the IR continuum.

In interpreting these data we must explain the clear intrinsic excess at wavelengths $\gtrsim1$ μm; four possibilities are investigated here.

(1) High-frequency continuation of the radio spectrum: SS433 is a variable (~factor 3) radio source[15,16] with a nonthermal spectral index varying between −0.6 and −0.48. With the flux levels quoted in the GHz range, even the most

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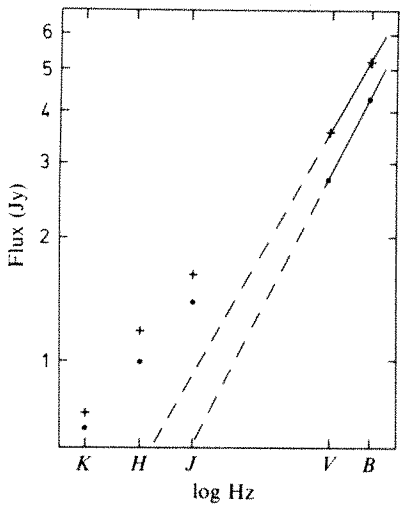

Fig. 1 Corrected fluxes from SS433 assuming that the interstellar extinction $A_v = 6$. Measurements taken on 19 (•) and 21(+) May 1979.

Table 1 Reduced fluxes observed from SS433* and phases

	May 1979 14	19	21
K	414 ± 38	420 ± 16	453 ± 13
H	420 ± 31	435 ± 16	518 ± 19
J	298 ± 65	364 ± 14	426 ± 24
V	11.9 ± 2.2	11.0 ± 0.6	14.1 ± 0.6
B	–	2.73 ± 0.40	3.38 ± 0.25
ϕ†	0.138	0.169	0.181

* Fluxes are given in millijanskys with errors of $\pm 1\sigma$.
† Phases computed after Martin *et al.*

optimistic extrapolation gives a flux ≤ 1.5 mJy at 1 μm, as opposed to the observed ~500 mJy. Hence some way of causing the nonthermal radio spectrum to flatten above 10 GHz must be found if this hypothesis is to be sustained; this does not seem to be the simplest explanation in view of possibilities (3) and (4) below.

(2) Circumstellar dust: this can be discounted almost immediately since to cause spectral flattening near 1 μm the dust would have to radiate at black body temperatures $\gtrsim$4,250 K, at which temperatures it could not survive. Also the absence of strong forbidden line radiation[1,2] implies that the gas mixed in with any dust must be fairly dense ($\gtrsim 10^8$ cm^{-3}) and would tend to destroy the dust by sputtering.

(3) A red stellar component: a black-body continuum at a temperature near 4,250 K will account for the spectral flattening near 1 μm as noted in (2) above. If this is due to a star its spectral type must be near K. Taking $A_v = 6$ and attributing the K magnitude of the object entirely to this hypothetical star we find $M_K \simeq -5.4$ at the usually assumed distance of 3.5 kpc (ref. 1). A colour $V - K \simeq 2.7$ appropriate to a K-type star gives $M_v \simeq -2.7$ at 3.5 kpc, so a star near spectral type K3II is indicated. Since M_v for the system is less than -4.5 with $A_v = 6$ the red star's spectrum would be completely unobservable in the optical. This explanation clearly requires the system to contain at least two components; a binary hypothesis is attractive if accretion on to a compact object turns out to be the underlying 'engine' for the relativistic gas motions. The main difficulty with this explanation of the IR data is the observed variation of the IR flux; apart from the steady increase reported here, Glass[7] observed variability of 0.8 mag at 2.2 μm. A steady variation might conceivably be due to long-period binary motion, but any more complicated variability[8] would probably have to be attributed to some other component of the system. If the variability is substantial this other component must supply most of the IR flux, so that an explanation of the spectral flattening would still be lacking.

(4) Free–free and free–bound emission from an optically thin gas: the continuum spectrum of such a gas at temperatures $\sim 10^4$ K would provide a good fit to the observed IR spectrum, allowing for some 'contamination' at short wavelengths due to the intrinsically steep optical continuum (if $A_v \gtrsim 6$). Evidence favouring this hypothesis comes from the great strength of Hα; with $A_v = 6$ the reddening near Hα implies an unattenuated flux of order 10^{-9} erg s^{-1} cm^{-2} in the three observed components[1,2]. If Hα is interpreted as a recombination line, as seems reasonable, this implies that the plasma emitting it produces a free–free continuum of order 500 mJy at frequencies $\nu \ll kT/h$, where T is the electron temperature of the plasma. (The ratio of the total strength of recombination Hα to the free–free continuum at such frequencies is almost independent of temperature). In other words, if Hα is produced by recombination, the continuum emission from the gas producing it will give an IR continuum of just the observed order if $A_v \simeq 6$. (Thus hypothesis (3) above would require also that Hα be produced from a predominantly neutral gas to explain the lack of observed free–free emission, unless A_v is assumed ≤ 4) If the IR flux $\simeq$500 mJy is interpreted as free–free emission from a gas near 10^4 K an emission measure $N_e^{\,2}V$ of order 10^{61} cm^{-3} is required, with only a weak dependence on the assumed temperature, assuming a distance of 3.5 kpc. The night-to-night variability in the IR implies a typical size $R \leq 10^{15}$ cm for the emitting region; with $V \sim R^3$ we see that the average electron density $N_e > 10^8$ cm^{-3}, accounting for the absence of observed forbidden-line emission. These limits imply a free–free optical depth $\tau_{ff} \gtrsim 10^{23}\nu^{-2}$, so the continuum will be optically thick and decline as ν^2 for frequencies $\nu < 10^{11}$ Hz; hence it will be unobservable in the radio region. To ensure that the continuum is optically thin at observed IR wavelengths implies $R > 10^{12}$ cm, $N_e < 10^{12}$ cm^{-3}. The emission measure 10^{61} cm^{-3} would imply a total cooling rate of order 10^{36} erg s^{-1}, much of which (in the absence of forbidden-line cooling) should appear as UV resonance lines. This cooling rate is of the same order as the observed 2–10 keV X-ray luminosity[6] (3×10^{35} erg s^{-1} at 3.5 kpc) and thus suggests photoionisation as the energy input to the emitting gas. As this is likely to lie far out from the central object, possibly near the ends of 'jets'[3,4], it would not intercept the line of sight to the X-ray source and hence not lead to an intrinsic photoelectric turnover in the X-ray spectrum. If photoionisation is the energy supply of the gas, the variability in the IR is readily explained by the variability of the central source, as observed in X rays.

We conclude that the IR emission from SS433 is most easily explained by free–free and free–bound emission from a region of gas with $10^{12} < R < 10^{15}$ cm, $T_e \sim 10^4$ K, $10^8 < N_e < 10^{12}$ cm^{-3} and a corresponding emission measure of the order of 10^{61} cm^{-3}, although an additional stellar component cannot be ruled out. More detailed speculation clearly requires both long term IR monitoring and some simultaneous IR–optical–X-ray observations.

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Infrared observations of SS433

David A. Allen

Anglo-Australian Observatory, P.O. Box 296, Epping,
N.S.W. 2121, Australia

The special features of SS433 have been chronicled over the past 12 months[1–4]. Most unusual of its features is the triple set of hydrogen and helium emission lines. Whilst one of these sets remains at rest, the other two exhibit velocity peregrinations of amplitude 0.27 *c*. These moving satellites have been interpreted[5,6] as outflowing gas in directly opposing jets which precess with a period of 164 days. I describe here observations of SS433 in the 1–4-μm spectral region made in March and April 1979 with the 3.9-m Anglo-Australian Telescope. These observations show that the Paschen and Brackett lines of hydrogen also exhibit moving satellites, substantiate the high reddening deduced from optical data, and indicate surprisingly strong free–free emission in the near IR.

All observations were made in daylight using the newly constructed IR photometer–spectrometer. Spectra covering the 2.0–2.5-μm region at a resolution ($\lambda/\Delta\lambda$) of 50 were taken on seven occasions from March 9 to April 15. All show emission features due to hydrogen. Within this spectral region only Bγ appears at zero redshift, at a wavelength of 2.17 μm. The moving satellite lines of Pα, Bβ and Bδ have also been identified, with the help of the ephemeris of Abell and Margon[5]. Table 1 lists numerical data on the spectra. Three of the spectra are shown in Fig. 1, and the motion of the satellite lines is evident from a comparison of the March 11 and April 6 data. In addition, the prominence of the satellite lines varies on a daily time scale, as can be seen from the March 9 and 11 data, and Table 1. Similar intensity fluctuations of the satellite lines are evident in much of the data (mostly unpublished) of various observers using the Anglo-Australian Telescope.

It would be of interest to compare the intensities of Bγ and the Balmer lines, and hence to derive a direct estimate of the reddening. So far, this comparison has not been possible due to the lack of concurrent optical data. The relative intensities of the rest and moving features appear to be much the same at visible and IR wavelengths, suggesting that all three gaseous regions experience similar reddening. The equivalent width of the Bγ line, which can be observed uncontaminated by satellite lines only when the latter are absent, on two of the seven spectra is 170 ± 30 Å; this figure may be overestimated by the inclusion of the strongest of the satellite lines (Pα) on the second occasion.

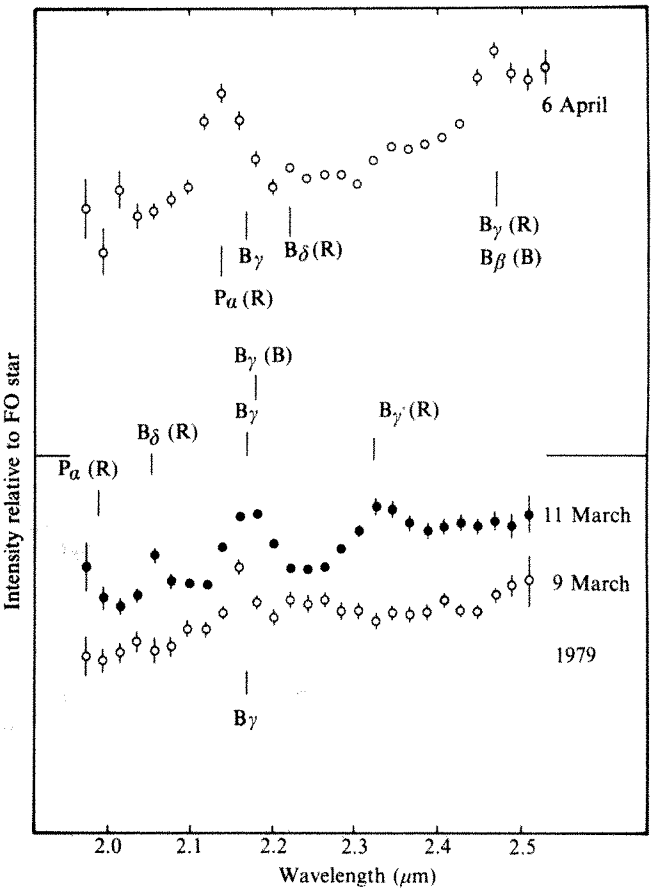

Fig. 1 SS433 2-μm spectra on three dates in 1979. Each spectrum was divided by that of the FO star ξ Ser to remove the telluric features, and all are reproduced at the same scale. The emission lines marked are the rest wavelength of Brackett γ, and the expected positions of the moving satellites. The latter are coded R and B according to whether the optical equivalents normally appear to the red or blue of rest.

Table 1 Observed emission lines in SS433 and their identifications

UT date 1979	Observed wavelength (μm)	Identification	Predicted wavelength (μm)	Equivalent width (Å)
9 March	2.16	Bγ	2.17	130
	no satellite lines present			
11 March	2.06	Bδ(R)	2.07	40
	2.17	Bγ	2.17	130
		Bγ(B)	2.18	
	2.33	Bγ(R)	2.31	100
6 April	2.14	Pα(R)	2.14	200
		Bγ	2.17	
	2.22:	Bδ(R)	2.22	10:
	2.47	Bβ(B)	2.46	120:
		Bγ(R)	2.47	
9 April	2.15	Pα(R)	2.15	230
		Bγ	2.17	
	2.41	Bβ(B)	2.43	100:
	2.48:	Bγ(R)	2.49	—
10 April	2.15	Pα(R)	2.16	240
		Bγ	2.17	
	2.41	Bβ(B)	2.42	80
	2.48	Bγ(R)	2.49	70:
13 April	2.16	Bγ	2.17	200
	no satellite lines present			
15 April	2.17	Bγ	2.17	240
		Pα(R)	2.18	
	2.39	Bβ(B)	2.39	100

Photometric observations were possible on only five occasions, due to the presence of cirrus at other times, and only on March 9 were data taken at all four available wavelengths of 1.25 (J), 1.65 (H), 2.2 (K) and 3.8 μm (L'). Over the month of observation the star monotonically brightened by half a magnitude at J, H and K. Of this increase, 0.3 mag occurred between 9 and 11 March (Fig. 1). The IR brightening has since halted according to the 1979 May data of Glass[7], and an abrupt fading by 0.2 mag between 14 and 15 April was reported by Milone[8].

It is necessary to correct the measured broad-band data for the influence of the emission lines. On 9 March the satellite lines were weak, and a reasonably accurate correction can be determined. Bγ contributes about 0.03 mag to the K datum. Using a reddening estimate of $A_V \sim 5$ (see below) and case B hydrogen line recombination ratios[9,10] for temperatures of the order of 10^4 K, the other corrections were estimated as follows: to J 0.03 mag due to Pβ at 1.28 μm, to L' 0.04 mag due to Bα at 4.05 μm. The corrected continuum magnitudes are: $J = 9.76 \pm 0.04$; $H = 9.10 \pm 0.02$; $K = 8.45 \pm 0.02$; $L' = 7.63 \pm 0.05$.

Two distinct estimates of the reddening experienced by SS433 have been attempted. One makes assumptions about the underlying star, which although plausible are not conclusive for such a controversial object. The other relies on a proved correlation between the reddening and the depths of certain unidentified absorption bands in the visible spectra of reddened stars. These estimates agree on a value for $A_V \sim 6$–7 (refs 1, 3). A third estimate can be derived from the IR photometry. This also makes assumptions about the underlying star, but the assumptions are perhaps more plausible than those pertaining to the optical region alone. In addition, the very presence of $B\gamma$ when $H\varepsilon$, which shares the same upper level, is too faint to have been observed indicates substantial reddening.

No mechanism is known at near IR wavelengths that produces a spectrum steeper than the Rayleight–Jeans tail of a black body. For the observed J–H colour, this imposes an upper limit of $A_V \leq 8$. Correction for $A_V = 8$ does not give a match to a Rayleigh–Jeans tail, in the sense that there is excess radiation at longer wavelengths. This we should in any case expect, due to free–free emission from the gas which produces the emission lines. A satisfactory fit can be achieved by a combination of a hot star and free–free emission for $4 \leq A_V \leq 6$. This also reproduces the observed (variable) visual magnitude of 13–14 (refs 3, 11) for K in the observed range 8.0–8.5. For $A_V = 5 \pm 1$, the contribution of the free–free component near $B\gamma$ can be estimated as 60±10%, and thus the equivalent width of $B\gamma$ on March 9 in terms of the free–free continuum is about 210±40 Å.

For gas temperatures in the range 5,000–20,000 K the expected equivalent width of $B\gamma$ relative to the free–free continuum[10,12] ranges from 900 to 400 Å. A value in this range was measured by the same equipment in the planetary nebula NGC 2440. An equivalent width of 210 Å implies an excessively high temperature; a better hypothesis is that an additional free–free continuum is present. One source of such a continuum is the gas producing the satellite lines. However, as the only measurements of the $B\gamma$ equivalent width were made when the satellite lines were weak, any free–free emission from the gas producing them would also be expected to be weak. Another possibility is that an abnormally high abundance of elements heavier than helium is present in one or all of the gaseous systems. The possible association of SS433 with a supernova remnant[1,2] lends some credence to this suggestion. Finally, a very small amount of dust radiating at a colour temperature of 1,000–1,500 K could provide the extra continuum required.

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H2155 − 304: a highly luminous BL Lac object

Philip Charles, John Thorstensen & Stuart Bowyer

Space Sciences Laboratory, University of California, Berkeley, California 94720

The radio source PKS2155 − 304 has recently been identified[1] with a HEAO 1 X-ray source and a ~14 mag object that has a featureless spectrum and is interpreted as a member of the BL Lac class. We describe here our optical spectroscopy of this object which reveals two weak emission features at λ5,820 and 5,870 Å. We suggest that these lines are [O III] λ4,959, 5,007 redshifted by $z = 0.17$. This result supports the interpretation of H2155 − 304 as a highly luminous BL Lac object.

The discovery of an intense soft X-ray source, H2155 − 304, at high galactic latitude[2] using the HEAO 1 A-2 experiment was followed by an accurate location with the A-3 modulation collimator[3]. This enabled a ~14 mag object to be proposed as the optical counterpart[1] which is within 1 arc s of the flat spectrum, point radio source PKS2155 − 304 (refs 4, 5). Optical spectroscopy of the candidate star[1,6,7] has shown that: (1) it is blue, $B - V < 0.1$; (2) it has a featureless spectrum, with upper limits to emission lines of 2–4 Å (ref. 7) and ~1 Å (ref. 1); and (3) it is variable in magnitude and colour on a time scale of months. Polarisation measurements[1] also showed a significant (~5%) wavelength dependent polarisation which decreased over ~3 months while the object brightened. The object was also found to be associated with a faint, asymmetric nebulosity[1]. This evidence is all highly suggestive of an elliptical galaxy whose nucleus contains a BL Lac object. Spectral features in BL Lac objects are extremely weak (if detectable at all)[8] as is evidenced by the difficulty in observing the emission lines from

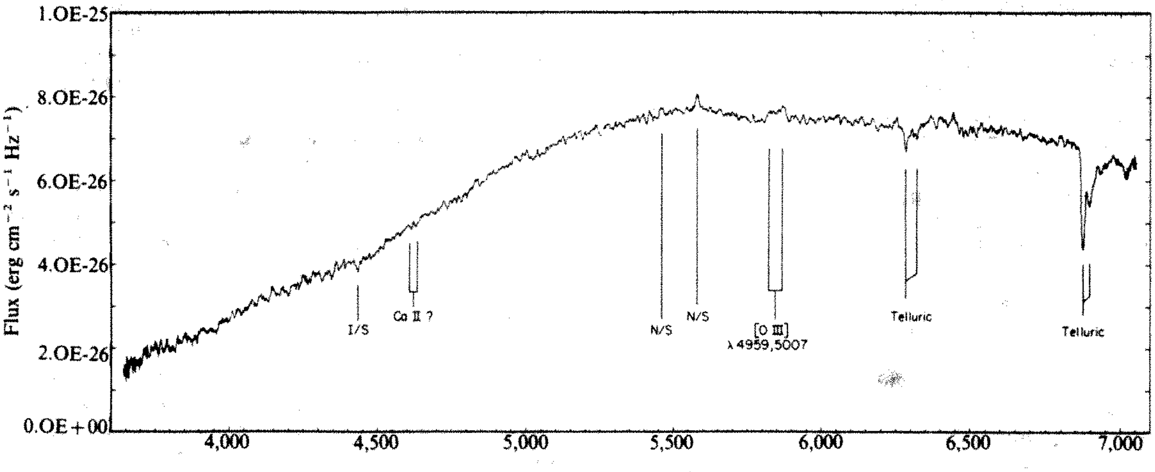

Fig. 1 Lick 3-m ITS spectrum of H2155 − 304 summed from three nights of observations in October 1978. The marked feature at ~λ 5,850 is identified with [O III] λ 4,959, 5,007 redshifted by $z = 0.17$. Features marked N/S are due to improperly subtracted night sky lines; the broad absorption bands are due to atmospheric O_2.

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Table 1 Comparison of properties of X-ray emitting BL Lac objects

Source	B		F			L_X(10^{46} erg s^{-1})			
		Ref.	(mJy)	ν(GHz)	Ref.	2–10 keV	Ref.	d(Mpc)	Ref.
Mkn 421	11.6–16.5	21	570	2.7	15	0.01–0.5	3	180	18
Mkn 501	13.5–14.5	16,17	1,450	2.7	15	0.016	3	200	18
PKS0548 − 322	14.9–16.1	16,20	310	2.7	4	0.06	3	410	19
2A1219 + 305	15.7–16.9	3	54	1.4	14	—	—	?	—
PKS2155 − 304	12.8–14.2	1	280	1.4	5	1.7	1	1,020	This paper

the nucleus of BL Lac itself[9] and the surrounding galaxy[10]. The optical spectroscopy of H2155 − 304 reported so far has either been of very poor spectral resolution (~40 Å)[7] or of limited sensitivity due to short observation time[1,6]. We therefore decided to undertake a series of observations of this object with the Lick Observatory 3-m Shane reflector and image tube scanner (ITS)[11] to acquire spectrophotometric data of high sensitivity. Our observations were made during the period 24–27 October 1978 and were reduced to an absolute flux level[12] by comparison with observations of standard stars[13]. Several integrations taken in good seeing conditions through relatively large (4 × 4 arc s) observing apertures, and guided so as to minimise the effects of atmospheric dispersion, yielded a mean V of 14.2 ± 0.1 and $B - V = 0.5 \pm 0.2$, substantially redder and fainter than the object appeared 6 weeks later[1]. The total mean spectrum of H2155 − 304 is shown in Fig. 1.

Of principal interest in this spectrum is the somewhat broad, double-peaked emission feature centred on λ5,850. Night sky features (due to imprecise sky subtraction) are marked N/S. If we interpret the emission feature as arising from two lines at λ5,820 and 5,870 we derive equivalent widths of 0.54 and 0.96 Å, weak, but certainly significant at this signal-to-noise ratio. Additional support for the reality of these lines comes from: (1) there are no night sky lines that, if improperly subtracted, could give rise to such a feature; (2) the lines are present on all three nights that this object was observed although at a reduced level of significance so that the feature is only statistically significant in the summed spectrum; (3) irregularities in the image tube would be extremely unlikely to produce such a feature because we adjusted the grating setting so as to cover a different wavelength range each night; thereby any real feature would move along the tube (as observed) whereas a tube 'hot spot' would not.

Comparison with the BL Lac spectrum[9] immediately suggests the identification of the H2155 − 304 lines with [O III] λ4,959 and 5,007. The implied redshift is $z = 0.17$ and hence $L_{\text{opt}} = 7.5 \times 10^{45}$ erg s^{-1}. At this redshift we have marked in Fig. 1 the implied position of Ca II H and K which might be present weakly in absorption (although there are other stronger features than this which are unidentified). Typically, these lines are easily resolved in active galactic nuclei with this instrument; in our spectrum, they are only marginally separated. An alternative identification for our feature, which circumvents this difficulty, is Doppler-broadened Hβ emission. However, the higher Balmer lines are not observed, rendering this hypothesis less attractive, unless the Balmer decrement is quite steep.

At this redshift H2155 − 304 is ~50 times brighter than BL Lac itself and in Table 1 the optical, radio and X-ray properties of the four definitely-identified X-ray emitting BL Lac objects are compared with H2155 − 304. The ~10 arc s nebulosity associated with H2155 − 304 has a physical diameter ~50 kpc at the Hubble distance of 1,020 Mpc (assuming $H_0 = 50$ km s^{-1} Mpc^{-1}) which, it has been pointed out[1], is not inconsistent with a large ellipitical galaxy. It would therefore be extremely valuable to use the technique of Miller *et al.*[10] to search for absorption lines in the galaxy spectrum and thereby (1) verify the redshift and BL Lac designation, and (2) investigate the stellar contribution to the nebulosity and compare it with those around QSOs. Given the variability of this object, its continued study spectroscopically is of considerable importance in the search for other lines and variability in the [O III] feature.

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A relationship between the amplitude of the susceptibility and the frequency of the maximum dielectric loss

R. M. Hill & L. A. Dissado

Department of Physics, Chelsea College, University of London,
Pulton Place, London SW6, UK

We recently presented a new theory of dielectric behaviour based on a many-body cooperative model of interacting dipoles[1]. It was shown that the experimentally observed non-Debye behaviour of many dielectric materials[2] can be represented by these interactions, and a relationship for the frequency dependence of the susceptibility which describes the observed behaviour was presented. The temperature dependence of the simplest dielectric materials which exhibit a dipole alignment transition has now been examined in detail[3]. One consequence of the cooperative theory is particularly novel and is reported here.

In a conventional dielectric the amplitude of the susceptibility is taken as proportional to the inverse temperature, or as proportional to the inverse of the difference between the temperature and the Curie temperature in a ferroelectric material. The frequency at which the loss peak occurs is simply activated, with the activation energy representing the potential difference between the meta-stable dipole potential minima and the maximum in the barrier between them[4]. It is known, however, that in the region of a phase transition, or the Curie temperature, that the frequency of the loss peak tends asymptotically to zero at the critical temperature[5]. The many-body

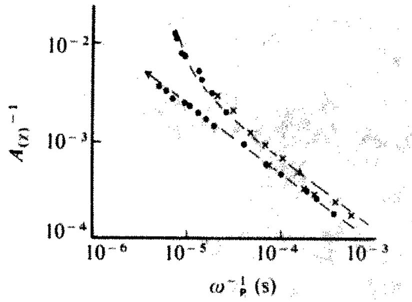

Fig. 1 A plot of the inverse of the amplitude of the susceptibility against the inverse of the frequency of maximum loss for $AgNa(NO_2)_2$ from the measurements of Gesi. Data from ref. 7 (○) and ref. 8 (×). The gradient of the parallel lines is −0.71.

theory[1,3] gives both of these features as a direct consequence of the basic assumptions. Furthermore the model predicts a set of intrinsic relationships between the magnitude of the susceptibility, $A_{(x)}$, and the peak frequency, ω_p. We have reinterpreted published data to show that one of these conditions has in fact been observed experimentally.

From equation (17) of ref. 1 the frequency at which the peak in the dielectric loss occurs is given by

$$\omega_p = \nu_e \cosh\left(\frac{B + kT_cM_e}{kT}\right)\{1-(1-M_e^2)\mathrm{T}_c/T\} \qquad (1)$$

The significance of the symbolism is given in that reference. From equations (27) and (31) of ref. 1 the magnitude of the susceptibility is proportional to

$$\omega_p^n \cos\left(\frac{n\pi}{2}\right)\zeta^{-n}M'_{(0)} \times F(\omega/\omega_p)$$

where $F(\omega/\omega_p)$ is the shape function normalised to ω_p. On substituting for the zero time value of the mean magnitude of the z component of the local unit dipole vector perturbation, $M'_{(0)}$, this expression can be written in the form

$$A_{(x)} = \left(\frac{\omega_p}{\zeta}\right)^n \cos\left(\frac{n\pi}{2}\right)\frac{(1-M_e)^2}{kT}\{1-(1-M_e^2)T_c/T\}^{-1} \qquad (2)$$

In the region of the critical temperature, T_c, the terms in parentheses in equations (1) and (2) dominate both the peak frequency and the amplitude of the susceptibility. Under this condition it follows that

$$A_{(x)} \propto \omega_p^{-(1-n)} \qquad (3)$$

Alternatively if the temperature T is not close to T_c the activated term in equation (1), ν_e, dominates and

$$A_{(x)} \propto \omega_p^n \qquad (4)$$

These relationships show the generality of the parameter n (ref. 6), which has been shown to be the measure of the degree of correlation of individual tunnelling dipolar transitions in the medium[1].

The properties of $AgNa(NO_2)_2$, which is a ferroelectric crystal with a low transition temperature (37.8 °C) have been reported by Gesi[7,8]. A compilation of these measurements is shown in Fig. 1, in which the inverse of the susceptibility amplitude has been plotted against the inverse of the peak frequency. The inverse functions have been used as the tracing technique for susceptibility normalisation described in ref. 2 results in temperature shift points which are these inverse functions. Figure 2 shows two parallel lines, one for temperature less than the critical value and the other for temperatures above T_c. The lowest temperatures investigated show a deviation away from the linearity condition towards the region of positive gradient contained in equation (4). The gradients of the lines are $-0.71 = -(1-n)$ and comparable to the equivalent value of $-(1-n)$ measured from the high frequency region of the normalised susceptibility/frequency plot for this material of 0.68. Similar results have been observed from Nakamura and Hosoya's measurements[9] on $Ca_2Sr(C_2H_5CO_2)_6$. In this case the magnitude of $(1-n)$ was 0.99.

We know of no alternative explanation for this specific interrelationship between the amplitude and the frequency of the maximum in the dielectric loss. Hence Fig. 1 presents direct experimental evidence for the validity of the many-body, cooperative, model proposed earlier.

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Stable ridges in a collapsing monolayer

Herman E. Ries Jr

Department of Biology, The University of Chicago, Chicago, Illinois 60637

The nature of monolayer collapse has long interested physical chemists studying surface phenomena[1–3], and is also of interest to biologists seeking cell-membrane models[4], since monolayers of fatty-acid type molecules are often used as models for each half of the biomembrane bilayers, and double layers or bilayers may well form on monolayer collapse. Furthermore thin-film properties of fatty acids are similar in many ways to those of cholesterol, lecithins, and other important components of biomembranes. There is little direct experimental evidence to support proposed collapse mechanisms, however. In this note a remarkable type of monolayer collapse is described. Pressure–area isotherms for 2-hydroxytetracosanoic (cerebronic) acid rise steeply and bend over sharply at a high pressure without the subsequent falloff in pressure expected for such rigid films. Electron micrographs of the collapsing film clearly show many folds or ridges of unusual stability. The compressed monolayer thus provides a model for membranes and thicker films under compression.

2-Hydroxytetracosanoic acid, obtained in high purity from Supelco Inc., was spread from a dilute benzene solution onto distilled water of pH 7.0. Pressure–area measurements were made using the Langmuir–Adam–Harkins technique[1,5]. Film samples were transferred to Formvar-coated electron microscope grids by a modified Langmuir-Blodgett method[6]. Transferred films were shadowcast in the direction of compression with platinum–palladium at an angle of 10° before examination in a Siemens Elmiskop 101 (ref. 7).

The data for duplicate determinations of the pressure–area isotherm are plotted in Fig. 1 and demonstrate the high reproducibility of the measurements. Extrapolation of the linear portion of the isotherm gives a cross-sectional area of 26 Å^2 per molecule. Monolayer compressibility is 0.0047 cm dyn^{-1} and the collapse pressure (without falloff) is 68 dyn cm^{-1}. Lycopodium tests demonstrate the extreme rigidity of the film. Compression-expansion-recompression experiments in the low-pressure region show negligible hysteresis. Water insolubility and surface mobility are thus established. [If any appreciable quantity of benzene is retained in the surface in our conditions it has little effect on the significant part of the pressure-area isotherm (the upper linear portion). These upper linear portions virtually coincide for benzene, hexane, and no

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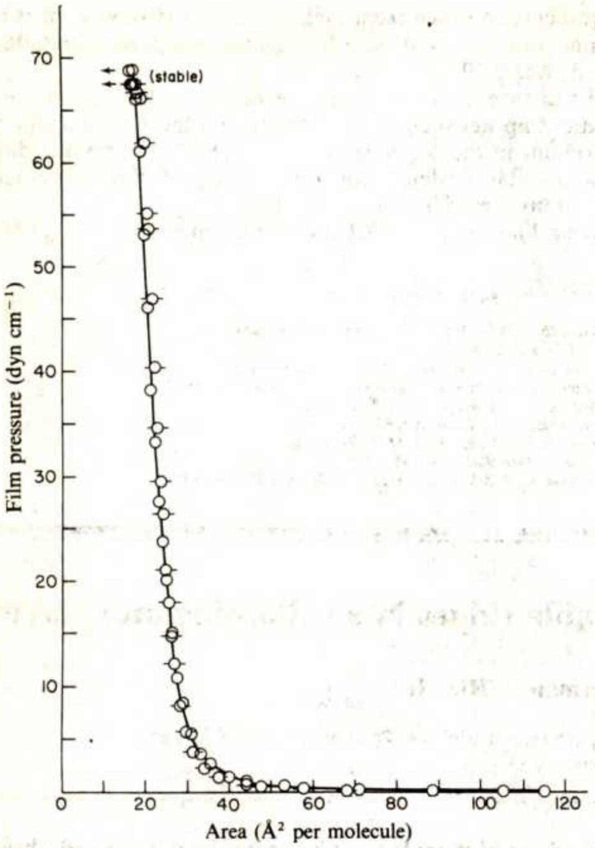

Fig. 1 Pressure-area isotherms for 1-hydroxytetracosanoic acid at 22 °C

solvent[8,9]. In contrast ethanol has a major effect on the isotherm[10].]

Essentially all rigid or solid-like films have previously shown a precipitous fall in pressure at the collapse or maximum pressure. A probable explanation is that such films break up into double-layer fragments as shown schematically in Fig. 2. Earlier electron micrographs of transferred films have supported this mechanism in which long narrow double-layer platelets form in four successive steps: weakening, folding, bending and breaking[3,11].

In Fig. 3, micrographs of the collapsing film of 2-hydroxytetracosanoic acid show essentially no broken fragments. Most striking is the high concentration of tall ridges or folds and the absence of fallen ridges or flat platelets. The ridges are oriented normal to the direction of compression. Many are approximately 2,000 Å in height or the equivalent of about 400 horizontally oriented molecules. In previous studies of solid-type films, such as those of fatty acids and cholesterol, only a few relatively isolated ridges were observed before the appearance of broken double-layer platelets[3,5,11]. The breaking off of these platelets from the monolayer proper probably accounts for the precipitous fall in pressure usually observed. Without the breaking off of the ridges in the case of 2-hydroxytetracosanoic acid, the monolayer continues to exert its maximum pressure.

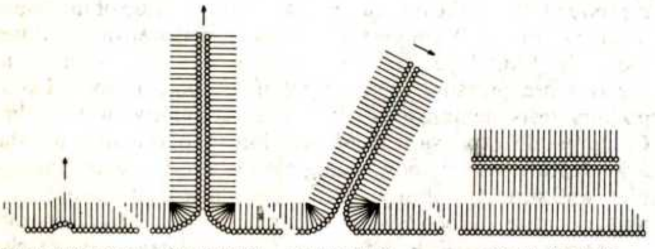

Fig. 2 Mechanism of monolayer collapse.

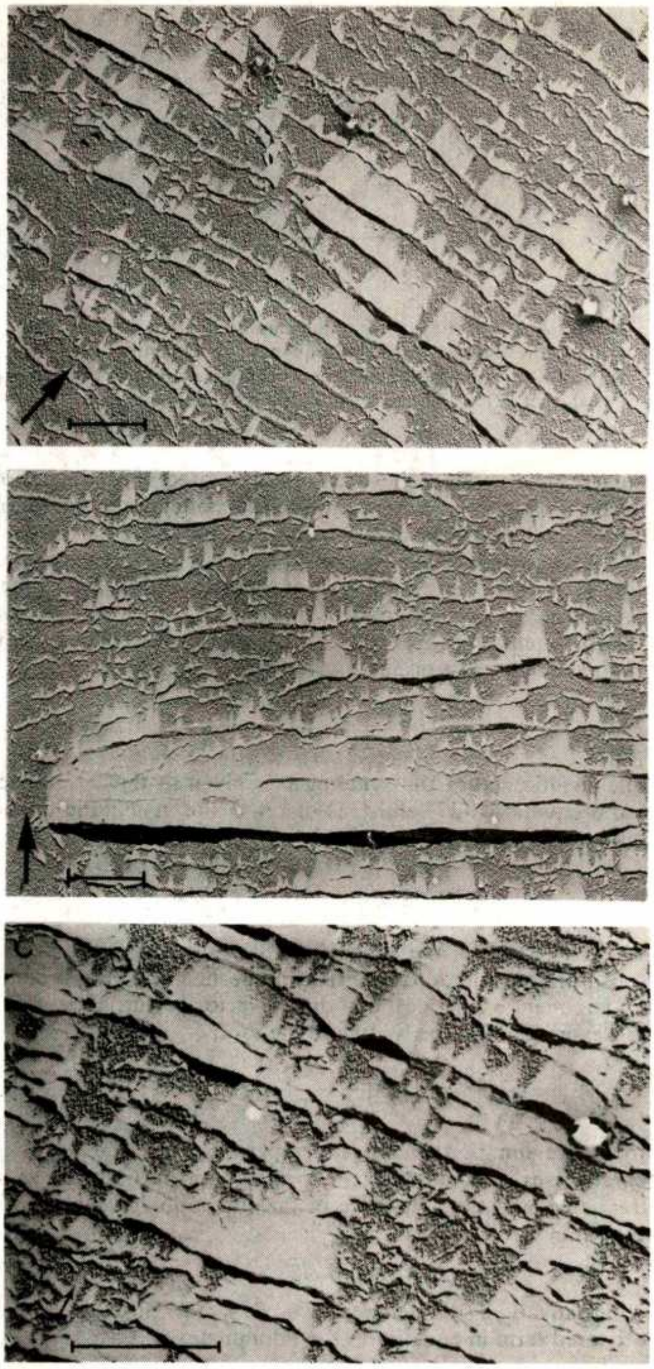

Fig. 3 Electron micrographs of collapsing films of 2-hydroxytetracosanoic acid. Arrows indicate the direction of shadowcasting and shadows appear as light areas. Scale bars, 1 μm.

Evidently the ridges have great stability in that they withstand the mechanical and thermal shocks of transfer, degassing and shadowcasting. Stability of the ridges and the remaining monolayer may well be provided by the cohesion of the 24-carbon atom chains combined with the intermolecular adhesion (H-bonding) of the hydroxy and carboxy groups. Furthermore, the ridges may provide a reservoir to replenish molecules lost from the monolayer. Collapsed or broken-off platelets would not provide a readily available reservoir because the polar groups of their component molecules could not easily contact the water surface.

Both the concentration and orientation of the ridges provide strong evidence that the compressed monolayer behaves much as a thicker sheet. The monolayer of 2-hydroxytetracosanoic acid may thus serve as an important model for cell membranes and other thicker film systems.

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Total gas content and surface elevation of polar ice sheets

D. Raynaud & B. Lebel

Laboratoire de Glaciologie du CNRS, 2, rue Très-Cloîtres,
38031 Grenoble-Cedex, France

Interpretation of stable isotope profiles from polar ice cores in terms of past climate requires a knowledge of the elevation at which the ice found at any depth was formed. Measurement of the total gas content of polar ice, *V*, can give such information if the volume of the pores in ice is known for the time they close off from the atmosphere at the ice formation site[1]. For the 1,387-m long core from Camp Century in north-west Greenland, estimates, based on the assumption of a constant pore volume at close-off, suggested that, on the average, ice from the Wisconsin/Weichsel period was formed at an elevation about 1,200–1,400 m higher than that from the Holocene[2]. This would account for an important part of the large shift in the stable oxygen isotopic ratio in this core between late Wisconsin and Holocene ice that was observed by Dansgaard and others[3]. However, if the pore volume at close-off depends on the site of origin for the ice, estimates of elevation of origin by the gas content method can be seriously in error unless the effect can be allowed for quantitatively. We present here results that enable us to describe the variations in the total gas content of ice that has been formed in present-day conditions in Antarctica and Greenland for a wide range of temperature (−12 to −53 °C) and elevation (400 to 3,200 m). Using these results, the gas content method can now be applied with much greater confidence than before to indicate past elevation changes.

The total amount of gas measured in ice formed by dry sintering of snow can be expressed as:

$$V = V_c \frac{P_c}{T_c} \frac{T}{P} \tag{1}$$

in which V is reduced to standard temperature T(K), standard pressure P, and 1 g of ice; V_c (per gramme of ice) is the pore volume when the pores are finally closed off in the firn at temperature T_c(K) and air pressure P_c. The conditions required for applying equation (1) are discussed in ref. 4. P_c and T_c depend mainly on the atmospheric pressure and temperature prevailing at the snow surface and, in the case of recently formed ice, they can be estimated from meteorological surface observations and temperature measurements in the drilled holes.

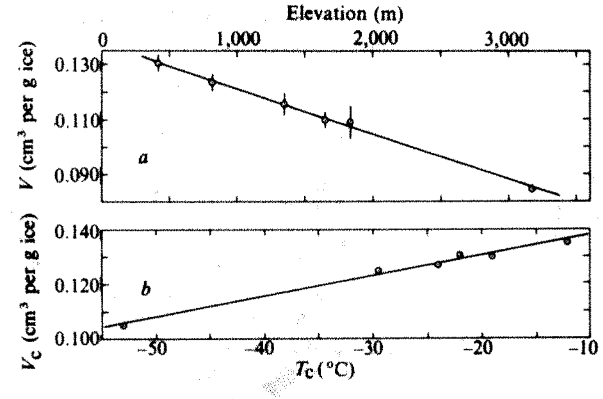

Fig. 1 Total gas content of Antarctic and Greenland ice versus the elevation of formation site *a*, and derived pore volume at close-off versus temperature at the formation site *b*. Standard deviations of the measured mean *V* values are indicated in *a*.

In order to analyse how the total gas content depends on present-day conditions, we have performed 142 determinations of V on ice recently formed in Antarctica and Greenland and recovered by drilling at six different locations (Table 1). The gas was extracted by melting the ice and refreezing the meltwater under vacuum, then dried and collected in a volumetric gas burette using a Toepler pump. Each measurement of V was performed on 20–25 g of ice, representing a thickness of 2.5–3.5 cm in the glacier. The relative precision of the measurements has been analysed from repetitive determinations of V on natural ice sampled from the same 2.5–3.5 cm thick layers. About 50 sets of such repetitive measurements were performed. The difference between these comparable measurements in V never exceeds 2% and is on the average 0.7%.

There are systematic differences, however, between results obtained using different procedures for extracting the gas under vacuum. For two locations, Camp Century and New Byrd, the current procedure yields mean V values (see Table 1) systematically 0.006 cm^3 per g of ice smaller than those previously reported by Raynaud[4]. This does not affect gas content changes in ice deduced from the results of a single procedure, and gas content changes obtained by different procedures may be compared.

Samples have been selected among the pieces of core available to obtain the best value for the amount of gas trapped when firn turns into ice under present-day and dry conditions. In particular, core measurements have been taken from unfractured sections that are apparently undisturbed by percolating meltwater and well below the pore close-off zone (since near this zone the pore seals may be delicate and consequently changes in the original gas content can occur between core recovery and measurement). At each drilling location, ice flow modelling results and isotopic profiles of the ice indicate that the layers investigated here were formed during the Holocene, when climatic conditions were similar to today and at approximately the same site. (At Dome Summit, Camp Century, and Dome C the investigated layers were most probably formed near the drilling site; but in the case of Cape Folger, D 10, and New Byrd the origin of the ice is considered to be different from the drilling location due to glacier flow and, in the case of New Byrd, to changes in ice thickness.)

The number of determinations of V performed for each site was between 5 and 58. Corresponding mean values with standard deviations are shown in Table 1. The variations observed for a given site are generally larger than experimental errors and are, in part, due to short-term natural fluctuations. The mean values of V show a significant and mostly proportional decrease

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with respect to the elevation (E) of the ice formation site (Fig. 1*a*). The least-squares linear regression is:

$$V(\text{cm}^3 \text{ per g of ice}) = -1.66 \times 10^{-5}\, E(m) + 0.138 \qquad (2)$$

with a correlation coefficient of −0.998 which is significant at a <1% level (Student's *t*-test).

Calculation of V_c using equation (1), the mean V values, and estimated values for P_c and T_c (Table 1), indicates that about half of the observed decrease in V with respect to E is linked to the change of the ratio P_c/T_c with elevation and that the remainder is due to variations in V_c. Any systematic change in the porosity at close-off must depend primarily on firnification parameters, such as the accumulation rate and the temperature which, in particular, influences the grain size. Our results indicate no significant correlation between accumulation rate and V_c. On the other hand, a very consistent dependence of V_c on T_c is obtained (Fig. 1*b*) which can be expressed by a linear regression:

$$V_c\,(\text{cm}^3 \text{ per g of ice}) = 7.4 \times 10^{-4}\, T_c(\text{K}) - 0.057 \qquad (3)$$

the level of significance of the correlation (coefficient = 0.992) being <1% (Student's *t*-test).

A further indication of a correlation between V_c and temperature arises from the short-term variations of V recorded by the two continuous sets of increments analysed from the D 10 and Dome Summit cores (Fig. 2). The amplitudes of these variations cannot be due only to experimental errors and fluctuations in P_c and T_c. They probably reflect natural fluctuations in V_c which contribute in a significant way to the observed V variations. The comparison between changes in V and isotopic composition (δD) in Fig. 2 suggests that ice formed from snow deposited in warmer conditions (high δ values) contains a higher pore volume (high V values) than ice formed from snow deposited in colder conditions (low δ).

The results shown in Fig. 1 lead to the following relation between V, P_c and T_c (obtained from equations (1) and (3)):

$$V\,(\text{cm}^3 \text{ per g of ice}) = \left[7.4 \times 10^{-4}\, P_c - 0.057\, \frac{P_c}{T_c(\text{K})}\right] \frac{T(\text{K})}{P} \qquad (4)$$

Thus the total amount of gas trapped in polar glaciers, when dry firn turns into ice, depends on the atmospheric pressure and temperature prevailing at the formation site. In present-day surface conditions, an elevation increase of 1,000 m corresponds to a decrease in V of about 0.017 cm^3 per g of ice. This gradient is close to those obtained over much smaller elevation ranges by Budd and Morgan[7] for the Law Dome (Antarctica) and from mean total gas content values reported by Miller[8] for stations Milcent and Crête (Greenland). According to equation (4), a general climatic cooling of 5 °C at the ice formation site would imply a decrease in V of approximately 0.001 cm^3 per g of ice. These figures clearly show the potentiality of V as elevation indicator and confirm that the mean difference of

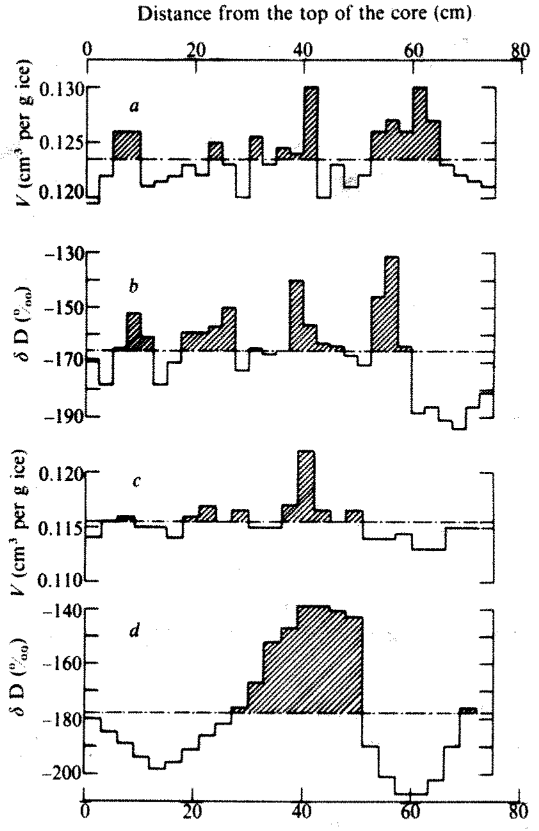

Fig. 2 Total gas and deuterium content variations observed on the two continuous sets of core increments taken at D 10 (depth range: 117.1–117.9 m below the surface), *a* and *b*, and at Dome Summit (232.0–232.7 m below the surface), *c* and *d*. The dashed lines are the average values.

Table 1 Mean total gas content, $\bar{V}$ measured on ice recently formed in Antarctica and Greenland

Drilling site	Depth interval (m below surface)	No. of determinations	$\bar{V} \pm \sigma$ (cm^3 per g of ice)	Elevation* (m)	P_c* (mbar)	T_c* (°C)	V_c (cm^3 per g of ice)
Cape Folger, F station (Law Dome, East Antarctica)	131.0–131.2	5	0.1305 ± 0.0025	420	935	−12	0.135
D10 (Terre Adélie, East Antarctica)	117.1–117.9	57	0.1235 ± 0.0030	820	895	−19	0.130
Dome Summit (Law Dome, East Antarctica)	100–233	58	0.1155 ± 0.0030	1,350	825	−22	0.1305
New Byrd (West Antarctica)	100–900	9	0.110 ± 0.003	1,650	795	−29.5	0.125
Camp Century (Northwest Greenland)	200–800	7	0.109 ± 0.006	1,840	795	−24	0.127
Dome C (East Antarctica)	155.0–155.2	6	0.0845 ± 0.0010	3,170	655	−53	0.105

The depth interval at which the ice has been taken as well as estimated elevation, atmospheric pressure (P_c) and temperature (T_c) of the ice formation location are also given for each site. V_c values are the calculated pore volumes at close-off from equation (1).

* From refs 4 and 5, except for New Byrd. For that site, according to ref. 6, the surface elevation at the origin site for the ice found now between 100 and 900 m depth is, on the average, about 150 m higher than the surface of the drill site, and P_c and T_c have been estimated taking into account a pressure–elevation gradient of 0.1 mbar m^{-1} and a temperature–elevation gradient of 0.01 °C m^{-1} for the Byrd Station area.

about 0.015 cm^3 of gas per g of ice measured between Holocene and Wisconsin ice in the Camp Century core[1] can be primarily attributed to an elevation effect. Taking into account the present-day V-elevation gradient (from equation (2)), the Wisconsin section of the core is found to be formed, on the average, at an elevation about 900 m higher than the Holocene section (which may be compared with the earlier estimate of about 1,200 m (ref. 2) which was based, among other things, on the assumption of constant V_c). The colder climate during the Wisconsin (compared with the Holocene) leads to a calculated elevation effect somewhat less; thus, if the climatic temperature change is 10 °C, the elevation difference would be about 800 m. Considering the present-day rate of decrease in stable isotope composition of ice with elevation of 0.62 δ^{18}O‰ per 100 m for Greenland[9], our new elevation estimate would account for about 5 δ^{18}O‰ or almost half of the mean isotopic difference between the Wisconsin and Holocene ice at Camp Century.

The gas content method, if extended to a wide range of polar ice cores, can provide unique information on ice origin and past thickness changes of polar ice sheets and thus enables more reliable interpretation of past climatic changes deduced from isotopic measurements on ice cores.

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Denitrification in the nitrogen budget of a river ecosystem

A. R. Hill

Department of Geography, York University, Toronto, Ontario, Canada M3J 1P3

Recent studies indicate that bacterial denitrification in anaerobic sediments may play a major part in removing nitrogen from water during river transport[1,2]. As this reaction reduces nitrate-N primarily to gaseous nitrogen, it represents a potentially significant pathway for the permanent removal of nitrogen from aquatic environments. However, no detailed assessments have been made of the quantitative importance of denitrification in relation to river nitrogen budgets. I have investigated this problem by studying the role of denitrification in relation to the nitrogen budget of Duffin Creek near Toronto, Ontario, and report here that the removal of nitrate-N by denitrification in the downstream reaches of the river represents 5–6% of the annual export of total nitrogen from this river basin.

Duffin Creek has an average flow of 2.2×10^5 m^3 d^{-1} and drains a predominantly rural watershed of 262 km^2. Nitrogen transport was measured at intervals along the two main branches of the river. River discharge measurements were available from recording gauges at sites 1, 2, 3, 6, 8, 11 and 13 (Fig. 1). At the remaining sites current meter measurements coupled with cross-sectional areas were used to calculate discharges at times of river water sampling. Nitrate-N was measured in water samples colorimetrically using an automated cadium reduction method[3]. Detailed measurements of all nitrogen forms were also available for sample sites used from government water quality monitoring studies[4].

During May to October in 1973 to 1975 and in 1978 river discharge and concentrations of nitrate-N were measured on 18 days of low flow discharges. A comparison of river discharges for each channel reach indicated that inputs and outputs of water differed by less than 15% for almost all the observations. The six river reaches examined showed a consistent pattern of daily losses of nitrate-N. The mean daily loss for the 18 days varied between 1.7 kg (40 mg m^{-2} of stream bed) and 20.3 kg (300 mg m^{-2} of stream bed) nitrate-N (Fig. 1). The concentration of ammonium-N and nitrite-N was negligible during low flow conditions. Calculations of organic-N transport revealed no evidence of significant gains or losses.

Uptake of nitrogen by benthic algae and macrophytes seems to account for ~15% of the nitrates removed from the river water (A.R.H., in preparation). The possibility that nitrate losses in Duffin Creek are caused mainly by denitrification in river sediments was investigated in a laboratory experiment. Sediment samples were covered with a solution containing 5 mg per l NO_3-N as KNO_3. Water samples were removed at various time intervals and analysed for nitrate-N. The results indicated a rapid disappearance of nitrate with almost total loss occurring in some samples within 6 days (Fig. 2). Average rates of nitrate-N loss over a 6-day period ranged between 100 and

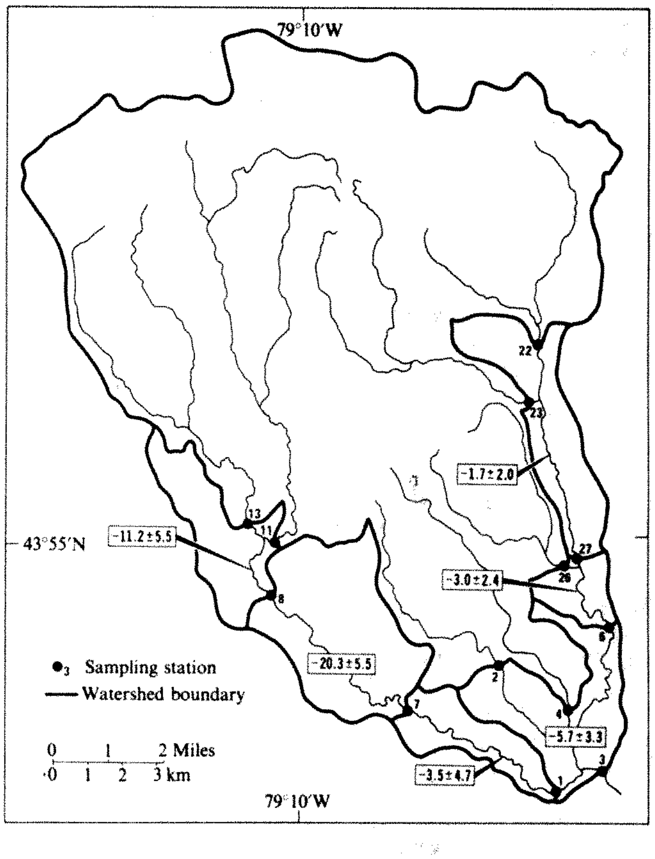

Fig. 1 Mean (±s.d.) daily losses of nitrate-N during low river flows in the late May–October period 1973–75 and 1978.

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251 mg m^{-2} of sediment surface daily for silt rich deposits and between 20 and 60 mg m^{-2} for samples with a high sand and gravel content. These rates of nitrate disappearance may provide an overestimate of denitrification losses because assimilatory nitrate reduction by bacteria was not evaluated. However, a recent laboratory investigation of sediments from streams in southern Ontario showed that 90–95% of the nitrate removal resulted from denitrification[5].

Nitrogen input–output balances were also measured for 10 days during the winter and spring months for the six downstream reaches of Duffin Creek. The data do not show any consistent pattern of nitrate-N removal, but this does not preclude the occurrence of denitrification. Small nitrate losses resulting from low rates of denitrification in cold temperature conditions would probably not be evident in the field data, owing to the range of error involved in the measurement of discharge and water chemistry.

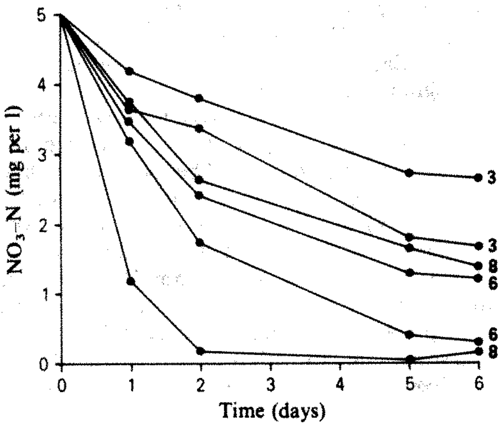

Fig. 2 Changes in nitrate-N concentration in water overlying sediment cores collected from sites 3, 6 and 8. The cores were incubated in the dark at a temperature of 20 °C. Air pumps were used to introduce bubles close to the water–sediment interface to reproduce the turbulence and aeration characteristic of river conditions.

The disappearance of nitrate-N was observed in 12 sediment–water profiles incubated at temperatures of 20 °C, 10 °C, 6 °C and 1 °C. From December to March the water temperature in Duffin Creek fluctuates between 0 and 2 °C. The laboratory data reveal that denitrification occurs in river sediments even at these low temperatures, although nitrate removal is only ~20% of the rate occurring at summer water temperatures which average 20 °C. River water temperatures range between 4 and 10 °C during November, April and early May. Water–sediment profiles incubated at 6 and 10 °C showed rates of nitrate loss which were 35% and 45% respectively of the nitrate removal at 20 °C. These results suggest that denitrification may occur in Duffin Creek during the winter and spring although at a much lower rate than is characteristic of late May to late October.

The field and laboratory data can be combined to calculate the annual removal of nitrogen by denitrification from the two main branches of Duffin Creek extending from sites 11, 13 and 22 to site 3 (Fig. 1). The 18 sets of field data collected between late May and late October indicate an average daily loss of ~45 ± 9 kg (s.d.) of nitrate-N for the combined six downstream river reaches. This daily rate of loss when adjusted to account for uptake by benthic algae and macrophytes would result in the removal of about 6,300 ± 1,250 kg of nitrate-N between 20 May and 31 October. Using the laboratory data on rates of denitrification at low water temperatures, it is estimated that an additional 2,000 kg of nitrate-N is lost between November and late May, producing a total annual removal of ~8,300 kg.

This calculation of annual nitrate-N removal ignores the possible effects of high river flows on denitrification. The occurrence of considerable disturbance and scouring of sediments during periods of storm flow probably creates aerated condtions which inhibit denitrification. The relationship between suspended sediment concentration and discharge at site 3 suggests that disturbance of the river bed sediment begins to occur at discharges greater than 2.83 m^3 s^{-1}. If it is assumed that denitrification is zero on days which have a mean daily discharge exceeding 2.83 m^3 s^{-1}, the annual removal of nitrate-N is reduced to about 7,300 kg.

The average annual export of nitrate plus nitrite-N from the Duffin Creek basin at site 3 for the period from May 1973 to May 1975 was ~73,000 kg (ref. 6). Measurements of Kjeldahl nitrogen concentrations are also available for the same period[4]. Calculations based on these measurements together with the daily river discharge records at site 3 indicate an average annual export of 62,000 kg Kjeldahl-N, giving a total nitrogen loss of 135,000 kg.

These observations allow an assessment of the importance of denitrification in relation to the nitrogen budget of Duffin Creek. The annual removal of 7,300–8,300 kg of nitrate-N in the downstream reaches of the river is ~5–6% of the annual export of total nitrogen from the river basin. This percentage must be regarded as tentative in view of the difficulty in estimating denitrification during the November to May period. Moreover, the budget technique used in this study cannot measure the denitrification of nitrate-N generated within bottom sediments: nitrates produced by nitrification in the oxidised surface layer of river sediment may subsequently undergo denitrification after diffusion to deeper anaerobic zones[7].

The importance of denitrification in relation to the nitrogen budget of Duffin Creek varies on a seasonal basis. Approximately 80% of the annual nitrogen exports from the river basin occur between November and April, when denitrification is negligible. However, denitrification is a major factor in the nitrogen budget of the creek from late May to October. Low river flows occur on approximately 140 days during these months each year. In these conditions there is an average daily removal of 45 kg nitrate-N in the downstream reaches principally due to denitrification. This loss represents 50% of the average daily input of total nitrogen and 75% of the nitrate-N input received by these channel reaches (A.R.H., in preparation).

The preservation or enhancement of denitrification in rivers has been viewed as a possible approach to the management of nitrogen in the environment[1,8]. These data suggest that this strategy would probably not produce a major reduction in the annual export of nitrogen from many drainage basins. However, benefits in the form of reduced nitrate-N concentrations in water and smaller nitrate exports to downstream ecosystems could be derived during low flow discharges in the warm seasons of the year.

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Anomalous propagation of seismic waves through the zone of shearing contact between converging plates of the New Hebrides arc

Remy Louat

ORSTOM BP A5 Noumea, New Caledonia

Jacques Dubois

ORSTOM 24, Rue Bayard 75008 Paris and Laboratoire de Geophysique, Université Paris sud 91405, Orsay, France

Bryan L. Isacks

Department of Geological Sciences, Cornell University, Ithaca, New York, 14853

Results obtained from a network of five short-period seismograph stations operated for 2 months on the island of Santo in the central region of the New Hebrides provide evidence for the anomalous propagation of seismic waves through the zone of shearing contact between the converging plates of the New Hebrides Island Arc.

Santo Island has an abnormal position in the subduction zone, being located where the trench is usually found. The crustal blocks including Santo and Malekula Islands separate the New Hebrides Trench into a northern and a southern segment (see Fig. 1). The evolution of this anomalous morphology, and the relationship of it to the subduction of a major aseismic ridge, the *D'entrecasteaux* Fracture Zone, remain as interesting problems[1-5]. However, recent studies[6-9] show that the overall configuration of the Benioff zone is not now significantly contorted or disrupted through the central region of the arc. The inclined zone of thrust faulting or shear contact between the subducted oceanic plate and the island arc appears to be continuous along the entire length of the arc and is located at very shallow depths beneath the island of Santo (Fig. 2).

Thus a network on Santo is in a unique position in relation to the plate boundary, a position attainable in most other subduction zones only by the deployment of ocean bottom seismographs. In particular, a station on the west coast of Santo is seaward of the shallow zone of seismicity in such a position that it receives seismic waves which must pass through the zone of shearing contact between the plates. Stations in eastern Santo receive seismic waves which propagate mainly above the zone of contact, so a comparison of records obtained on Santo can show large anomalies in seismic wave propagation that might be associated with the zone of plate contact.

Five stations were operated on Santo as shown in Fig. 2. Earthquakes were located from arrival times of P and S waves. The accuracy of location is about ±5 km. The station on the

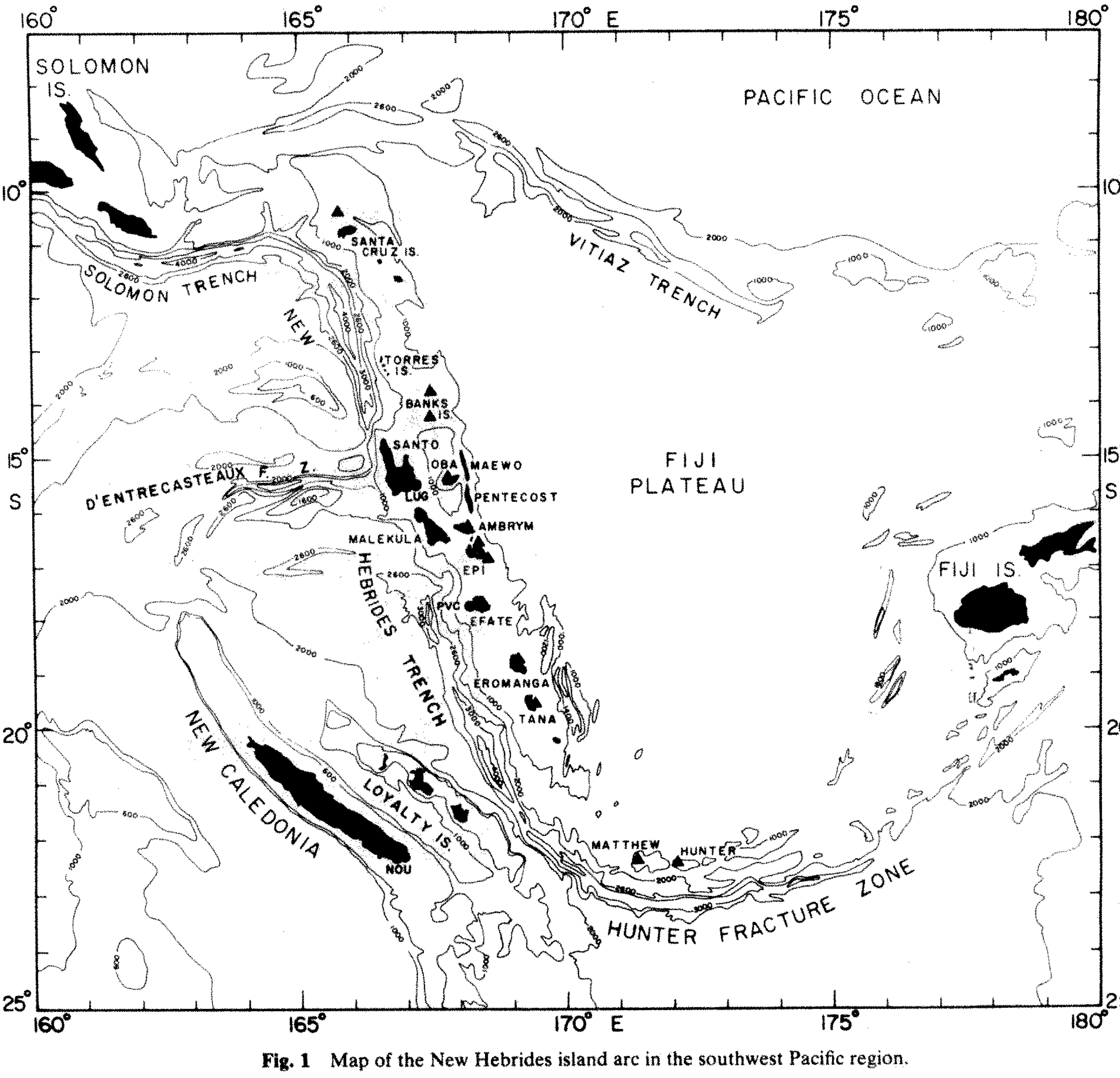

Fig. 1 Map of the New Hebrides island arc in the southwest Pacific region.

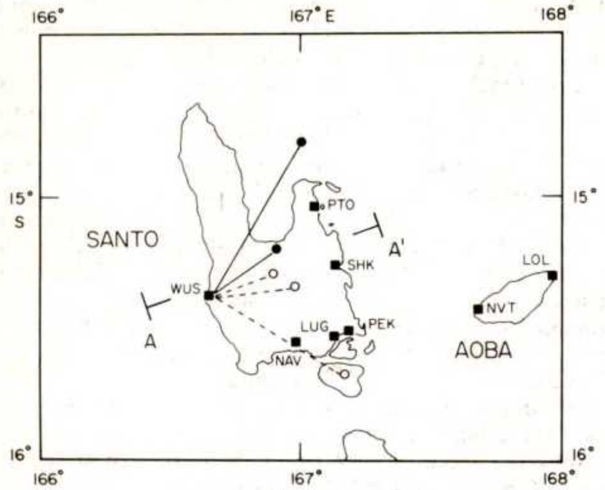

Fig. 2 Map of Santo and Aoba Islands showing temporary seismograph network operated in 1975. The three-letter abbreviations and solid squares indicate the seismograph stations. The solid and open circles show epicentres of the numbered earthquakes shown in Figs 4 and 5. Schematic ray paths to the station WUS are shown from those events. The dashed ray paths indicate low frequency shear waves at WUS.

west coast of Santo, WUS, operated for 10 days. The results for the period when WUS was recording are illustrated in Fig. 3. The dashed line is an approximation of the upper limit of the descending plate (see ref. 9). First motions of P waves from the cluster of events shown at shallow depths in Fig. 3 also indicate that the cluster is within the zone of plate contact. Events such as numbers 26 and 81 (Fig. 4) are reliably located beneath the thrust zone and are within the descending plate.

Although WUS recorded numerous events located beneath the thrust zone at intermediate depths, it clearly recorded only three of the events within the thrust zone (Fig. 3). As shown in Fig. 4, for two of the events (nos 79 and 88) the shear waves recorded by WUS have a frequency content remarkably lower than those from the same earthquakes recorded by stations PEK and SKB located in eastern Santo. The third event (no. 74) produces no clear shear waves at WUS, although the P wave is

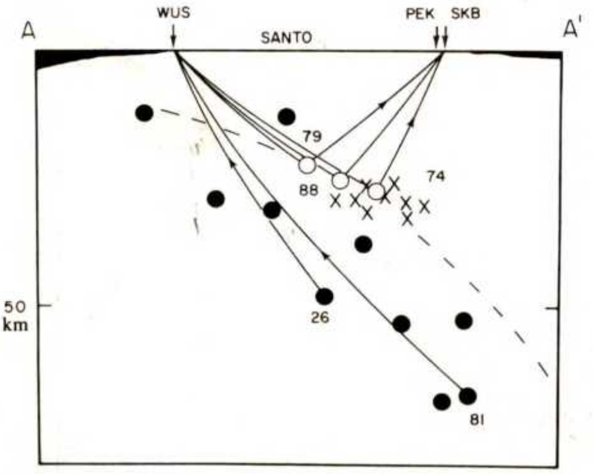

Fig. 3 Vertical section (see Fig. 2) showing events located during the 10-day period during which WUS was in operation. Crosses indicate events not perceptible on the WUS records, and circles indicate events recorded by WUS with (filled circles) or without (unfilled circles) high frequency shear phases. The ray paths are schematic and are not the result of quantitative ray tracing.

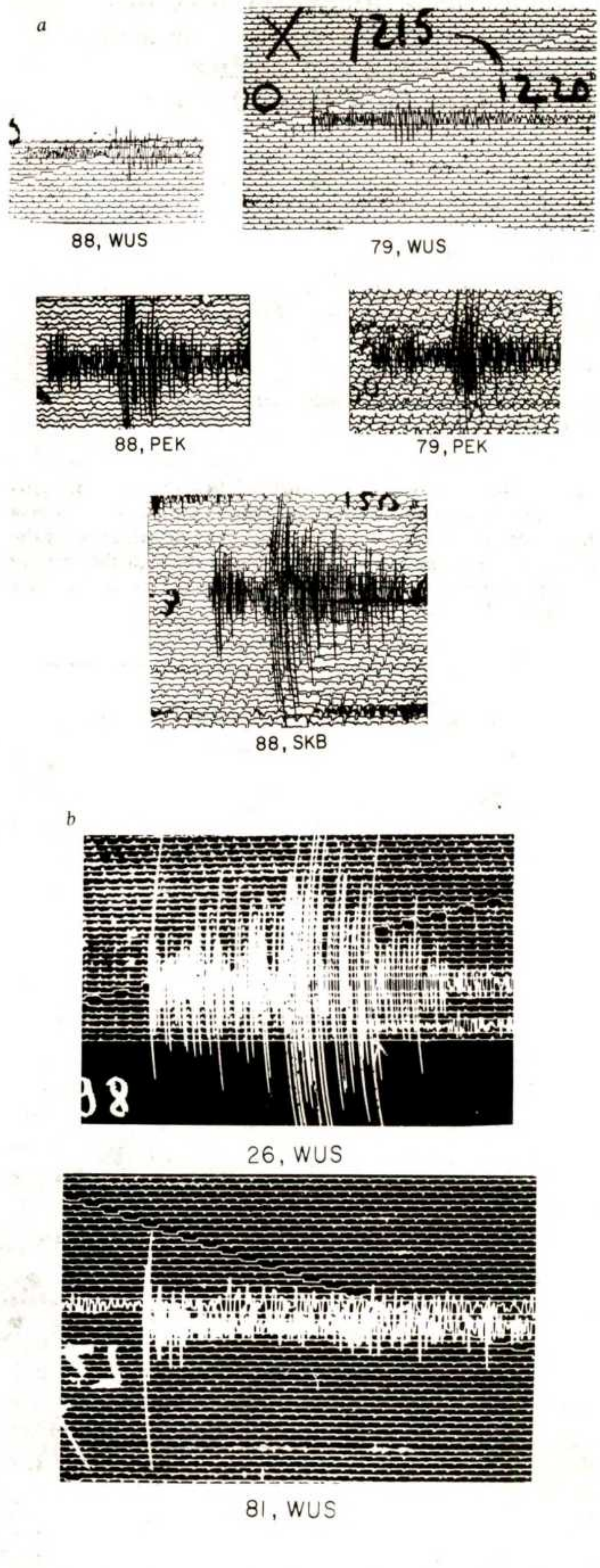

Fig. 4 *a*, Examples of seismograms from stations on Santo. The numbers and stations correspond to those shown in Figs 2 and 3. Second marks are clear on all records; the longer marks in certain records (for example, no. 79, WUS) are minute marks. *b*, Same as *a*, but showing different seismograms.

well recorded there, and high frequency shear waves are well recorded for this event in eastern Santo. In contrast, WUS records high frequency shear waves from events not within the shear zone (for example nos 26 and 81, Fig. 4), as do the other stations on Santo. The low frequency shear waves recorded by WUS for the two events in the thrust zone have predominant frequencies of 2 to 2.5 Hz, whereas the shear waves recorded by WUS for other events and by other Santo stations typically have predominant frequencies of 4 to 10 Hz.

Although the data are few, the combination of paths shown in Fig. 4 indicates that the unusual low frequency shear waves are not likely to be a localised effect near WUS. The anomalies must be a result of either a very special and unusual azimuthal variation in the radiation from the source, or, more likely, an effect of the propagation path. Results obtained so far raise the possibility that the shear waves to WUS suffer anomalously high attenuation within the zone of shearing contact between the converging plates of the subduction zone. The rays from earthquakes within the thrust zone must travel most of the way to WUS through or near the thrust zone. In contrast, rays from other events to WUS or from events within or beneath the thrust zone to other stations on Santo must only cross obliquely or pass above the thrust zone. If the removal of high frequency waves along the paths to WUS is by non-elastic dissipation, it may be possible to relate this to the development of a zone of viscous slip which is thermally activated by shear heating (as discussed by Yuen *et al.*[10]). However, Yuen *et al.* consider that such a zone would probably develop at greater depths and further down along the dip of the plate boundary. Alternatively, the attenuation may reflect scattering associated with heterogeneities developed within a zone of intense shearing. In either case, the zone should have a width sufficiently large to affect the passage of seismic waves.

Clearly further observations must be obtained. However, the data presented here suggest that useful information about the physical properties of a major earthquake-generating plate boundary may be obtained from suitably deployed local seismograph networks.

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A Lower Carboniferous *Lepidodendropsis* flora in Kashmir

A. K. Pal

Geological Survey of India, 27 J.L.N. Road, Calcutta, India

W. G. Chaloner

Botany Department, Birkbeck College, University of London, Malet St, London WC1, UK

The concept of a more or less uniform world-wide flora (the '*Lepidodendropsis* flora') of early Carboniferous age, was first expounded by Jongmans[1]. He cited the fossil plant genera *Lepidodendropsis*, *Triphyllopteris* and *Rhacopteris* as characterising this palaeofloristic unit, of which he claimed representation from a number of Northern and Southern Hemisphere localities. Subsequent work has generally confirmed his conclusion that in the early Carboniferous a floristically rather uniform lycopod-rich association extended into all the major continents, preceding the onset of Carbo–Permian glaciation. In its relative uniformity this flora contrasts sharply with the pronounced regional character of the floras (especially the Gondwana *Glossopteris* flora) that followed the glaciation. Although representation of Jongmans' *Lepidodendropsis* flora was claimed for the major southern land masses (South America, Africa, Australia) only a very limited Carboniferous flora, of a few genera of fern/pteridosperm leaves, has been reported[2,3] from India. This flora, from the Thabo stage of Spiti, in the Western Himalayas, was the only Indian Carboniferous flora known hitherto, and it differed significantly from its counterparts in other parts of Gondwanaland in the absence of *Lepidodendropsis*, and indeed any other lycopods. A newly discovered flora of Lower Carboniferous age from the Gund Formation of Kashmir, some 400 km west-northwest of Spiti is reported here.

The Gund Formation comprises about 500 m of cyclic sediments consisting of conglomerates, sandstones and shales. It overlies unconformably the Syringothyris Limestone, regarded as of Tournaisian age on faunal grounds, and is overlain by the Fenestella Shale Formation of Upper Visean to Bashkirian age. It is thus securely bracketed as lying at least within the Lower Carboniferous[4].

The Gund flora contains several genera which link it with typical *Lepidodendropsis* floras from other parts of the world; it is dominated by a number of lycopods, particularly *Lepidodendropsis* (Fig. 1*b*), *Lepidosigillaria* (Fig. 1*c*), *Archaeosigillaria* and ?*Cyclostigma*, together with *Archaeocalamites* (Fig. 1*a*), *Rhacopteris* (Fig. 1*d*, *e*) and other sphenopterids. In these constituents the Gund flora shows significant agreement with the Early Carboniferous *Lepidodendropsis* flora from Europe, from the Pocono Formation of the US, and floras of comparable age in Peru, Ghana, Morocco, eastern Sahara and Egypt, Syria, China and western Malaysia. The common elements are shown in Table 1.

Jongmans[1] regarded his *Lepidodendropsis* flora as "the flora of the older part of the Lower Carboniferous and as the transition to the *Archaeopteris–Cyclostigma* flora of the true Upper Devonian". Note that in the Lower Carboniferous of Geigen Hof, Bavaria, where Lutz[5] first recognised *Lepidodendropsis hirmeri* (the type species), this genus occurs in association with *Rhacopteris* and various other sphenopterids, together with *Sphenophyllum*. Similar, more or less contemporaneous, floras are found in many parts of Europe with minor variations, such as the *Lepidodendropsis–Archaeocalamites–Rhacopteris* association of North Wales[6] or the *Lepidodendropsis–Archaeocalamites–Triphyllopteris–Rhodeopteridium* association of southern Spain[7]. Floras of the Pocono Formation in Pennsylvania and Virginia are strikingly similar to these, and comprise various species of *Lepidodendropsis*, *Cyclostigma*, *Triphyllopteris* and other sphenopterids[8]. A *Lepidodendropsis–Adiantites–Fryopsis* flora is known as far north as Alaska[9].

Comparable assemblages occur on the now separate parts of the Gondwanaland mass. A *Lepidodendropsis–Cyclostigma–Triphyllopteris–Adiantites–Rhacopteris* assemblage is known from Carhuamayo in Peru[10]. In Africa, *Lepidodendropsis* is reported from Ghana[11], and from Morocco[12] through Libya[13] and the eastern Sahara[14] to Egypt and Sinai[15,16], in association with one or more of the genera *Prelepidodendron*, *Archaeosigillaria*, *Lepidosigillaria*, *Archaeocalamites*, *Sphenopteris* and *Rhacopteris*. The early Carboniferous age attributed to Australian reports of *Archaeocalamites*, *Lepidodendron*, *Rhacopteris*, *Fryopsis* and *Adiantites* has been questioned[17]. In south-east Asia a *Lepidodendropsis–Sublepidodendron–Archaeocalamites–*

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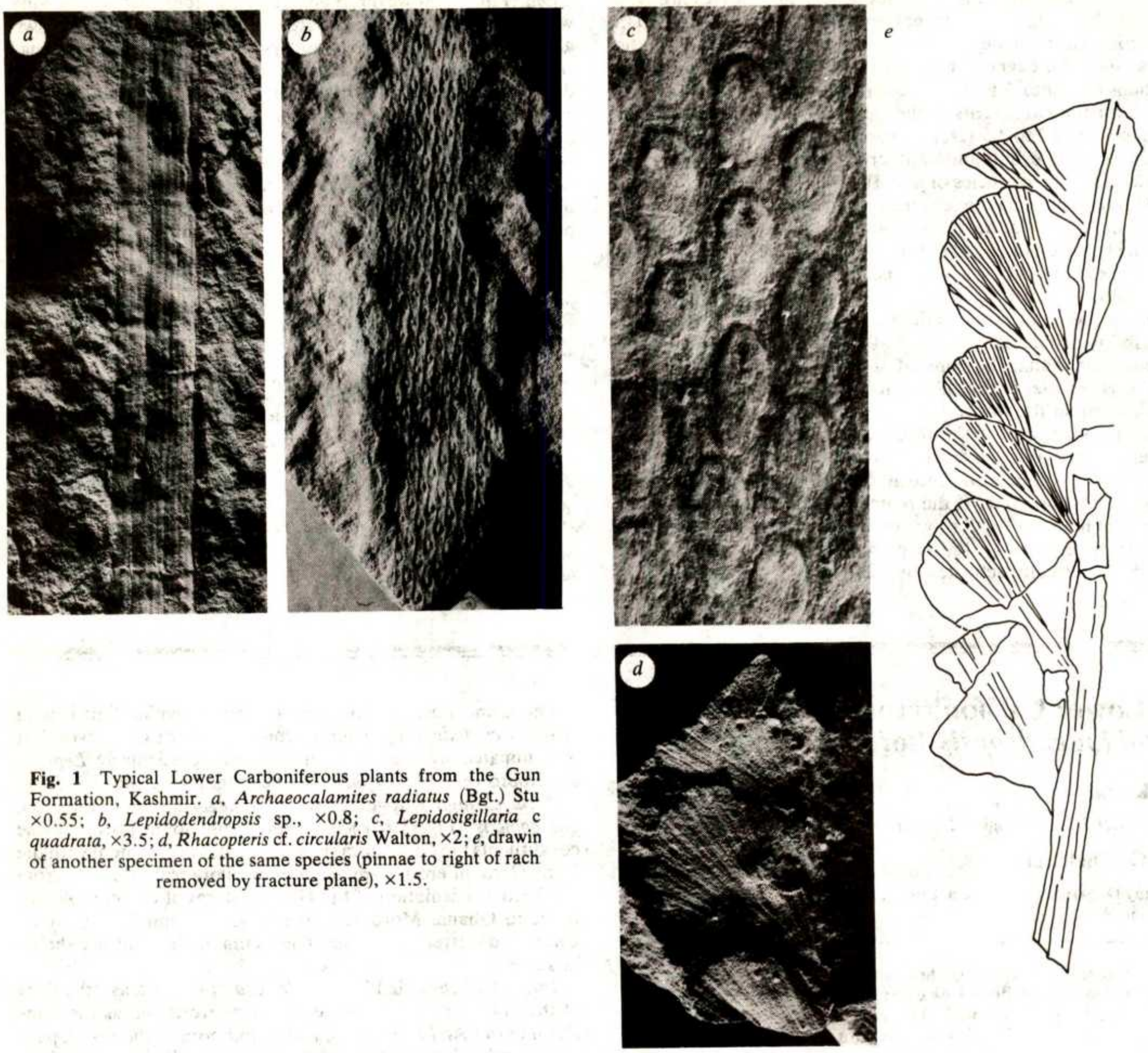

Fig. 1 Typical Lower Carboniferous plants from the Gun Formation, Kashmir. *a*, *Archaeocalamites radiatus* (Bgt.) Stu ×0.55; *b*, *Lepidodendropsis* sp., ×0.8; *c*, *Lepidosigillaria* c *quadrata*, ×3.5; *d*, *Rhacopteris* cf. *circularis* Walton, ×2; *e*, drawin of another specimen of the same species (pinnae to right of rach removed by fracture plane), ×1.5.

Table 1 Common features of the Gund flora and early Carboniferous floras found elsewhere

Fossil plants in the Gund Formation	Europe (early Carboniferous)	North America (early Carboniferous)	Peru (early Carboniferous)	North & West Africa (late Devonian to early Carboniferous)	China (early Carboniferous)	Malaysia (early Carboniferous)
Lycopsida						
Archaeosigillaria sp.	*			*		
Lepidosigillaria cf. *quadrata* Danze–Corsin (Fig. 1*c*)				**		
Lepidodendropsis sp. (Fig. 1*b*)	*	*	*	*	*	*
Lepidodendropsis cf. *sigillarioides* J.G. & D.	*	**	*	*	*	*
Lepidodendropsis cf. *fenestrata* Jongmans	*	*	*	**	*	*
'*Cyclostigma*' *ungeri* J.G. & D.	*	**	*	**		
Pteropsida						
Rhacopteris cf. *circularis* Walton (Figs. 1*d*, *e*)	**	*	**	*		
Rhodeopteridium tenuis Gothan	**	**			*	*
Sphenopsida						
Archaeocalamites radiatus (Bgt.) Stur (Fig. 1*a*)	**	**	*	*	**	

** Species in common; * Genus in common.

Triphyllopteris–Cardiopteris association is known from Wutung in Kianshi Province, China[18,19]. Further south a *Lepidodendropsis–Lepidodendron–Adiantites–Rhodeopteridium* assemblage occurs in the Lower Carboniferous of western Malaysia[20].

In the area east of the Urals floras occur that are characterised by several endemic genera of lycopods (*Ursodendron, Tomiodendron, Lophiodendron*) together with *Angaropteridium* and *Chacassopteris*[21]. While the separation of these several Russian genera may well be justified, the general aspect of these Lower Carboniferous Angara floras is broadly comparable to contemporaneous floras from other parts of the world[9].

Almost all known records of *Lepidodendropsis* are Lower Carboniferous in age, apart from two species reported from the Devonian of China[22], and the reports by French workers of this and other lycopod genera from the Devonian of North Africa. The reports from North Africa of several genera (*Lepidodendropsis, Archaeosigillaria, Tomiodendron, Abacodendron*) in rocks of Devonian age, considerably pre-dating their first occurrence elsewhere, has led to the suggestion[23] that Africa was the centre of origin of this whole group of arborescent lycopods (Lepidodendrales *sensu lato*). Until the age of the African material can be linked more securely with reliably-dated marine faunas this interpretation must be regarded as controversial.

The age of the Australian *Rhacopteris–Lepidodendropsis–Cyclostigma* flora, originally regarded[24] as Lower Carboniferous has more recently been reinterpreted as of Upper Carboniferous (Westphalian) age[17]. Such uncertainties in the age of pre-*Glossopteris* floras in Gondwanaland emphasise the significance of a flora such as that of the Gund Formation, in lying within a sequence containing marine faunas above it and below.

The Gund flora reported here confirms the close comparability of the Lower Carboniferous flora of northern India with that of other areas both in Gondwanaland and the (present) Northern Hemisphere. The four well-explored parts of the Gondwana block (India, South America, Africa and Australia) now prove to have had as close a floristic similarity before the Carbo-Permian glaciation, as that shown by the more intensively studied *Glossopteris* flora which succeeded it. A more detailed comparison of the plants of the Gund flora with those from other parts of Gondwanaland will be presented elsewhere.

Recent palaeomagnetic data from the Silurian and Devonian of China[25], has led Keppie[26] to divide Eastern Asia into two parts along a line through the Tien Shan-Mongol mountain belts to Vladivostock in his palaeogeographic reconstruction for the Devonian and Early Carboniferous. He was influenced in this proposal by the otherwise puzzling juxtaposition in the same latitudes of the Early Carboniferous Angara floral province with that of *Lepidodendropsis* in China. His reconstruction now places the boundary between these provinces at a palaeolatitude of approximately 35° N.

The present-day Chinese and Malaysian areas are brought in Keppie's reconstruction[26] into closer proximity to Gondwanaland than generally visualised hitherto, leaving their *Lepidodendropsis* flora disjunct from that of the main body of Eurameria. A somewhat more westerly position for the Sino-Malaysian block, possibly lying north and west of Australia, would offer a continuity with the *Lepidodendropsis* floras of Africa, South America and Europe, with this newly-discovered flora in Kashmir acting as a link between the eastern and western occurrences of *Lepidodendropsis*.

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A gliding reptile from the Upper Permian of North East England

T. H. Pettigrew

Sunderland Museum, Borough Road, Sunderland, Tyne and Wear, UK

In July 1978 an unusual fossil reptile was discovered by D. Hall and myself 1.5 cm above the base of the Upper Permian Marl Slate at Eppleton Quarry near Hetton-le-Hole, Tyne and Wear (NZ 351484). The sapropelic Marl Slate, the first member of the English Zechstein succession, is a marine deposit well known for its fossil fish (mainly palaeoniscids) and land-derived plants[1]. The only recorded reptilian remains consist of two incomplete skeletons of *Protorosaurus*[2]. The new find is an incomplete skeleton (the skull, forelimbs and most of the tail have been destroyed by quarrying), 17 cm long, of a terrestrial reptile washed into the Zechstein Sea. It is especially interesting for its elongated and jointed dorsal ribs interpreted as an adaptation for the support of two membranes to enable the reptile either to parachute or more probably glide in a manner similar to the modern gliding lizard *Draco* of South East Asia[3].

The reptile is lying on its back and the bones, severely crushed by compaction of the clay matrix, are very fragile. The axial skeleton consists of 36 amphicoelous vertebrae comprising 13 constricted dorsals, 2 sacrals and 21 slightly constricted caudals. On the left side there are 21 elongated ribs, the last six of which articulate with the first six dorsal vertebrae. The remaining 15 ribs converge anteriorly towards the vertebral column until truncated by the edge of the rock slab in which the skeleton is embedded. From this it is evident that at leat 15 anterior dorsal vertebrae are missing, and an original pre-sacral count of at least 28 vertebrae is indicated.

All the ribs have a prominent ventral groove and the rib heads are weakly dichocephalous, articulating dorsoventrally with the centra by means of narrow 'shoulders'. Because they lie closely packed together and are partially superimposed, it is difficult to determine the relative lengths of the elongated ribs, although anteriorly they measure at least 130 mm. Several important features are visible on the left side, where each rib is interrupted by a break interpreted as a joint near the rib head (Fig. 1). These joints divide the ribs into a relatively short proximal end (articulating through the rib head with the vertebral column) connected (through the joint) to an extremely attenuated distal end tapered to a hair-like extremity. On the right side this relationship is almost obscured by the attenuated distal rib ends which are tightly folded back against the vertebral column (Fig. 1). The joints probably enabled the distal ends of the ribs, supporting the 'wings', to articulate in a horizontal plane either

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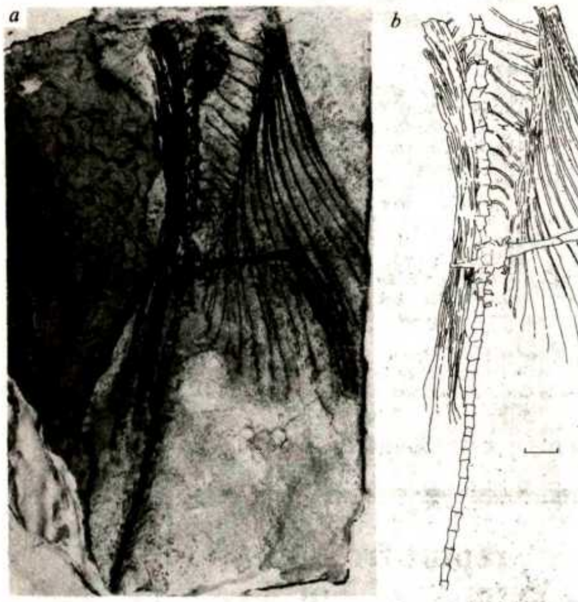

Fig. 1 Photograph (*a*) and sketch (*b*) of the reptilian skeleton from the Marl Slate of Hetton le Hole. Scale bar, 1 cm.

to spread them for gliding or to fold them back against the body for resting or walking. The thin, sinuous character of these ribs suggests they must have been fairly flexible and it is likely that, as in *Draco*, they were supported and pulled into shape by an elaborate arrangement of muscles and ligaments[3]. Behind these ribs are four short ribs also characterised by weakly dichocephalous rib heads.

Although the pelvis is crushed, a posteriorly directed iliac blade is visible. The left hind limb comprises a femur (27 mm long), fibula (18 mm long), tibia (19 mm long) and an elongate, clawed pes of which the tarsus and five digits have been crushed and splintered. The femur, tibia and fibula are relatively slender and thin walled. Of the right hind limb, preserved in counterpart, only fragments of the femur and an almost complete pes are preserved. Four broad-headed metatarsals are each 6 mm long. The fourth digit is 22 mm long with a deep and narrow claw. Several such claws are visible at the extremities of the digits of both right and left pes.

The Upper Permian Kupferschiefer is the European equivalent of the Marl Slate and in Germany has yielded three fragmentary skeletons of gliding reptiles. Only one, the holotype of *Weigeltisaurus jaekeli*, has been fully described[4,5], consisting of a well preserved skull with a partially disarticulated skeleton. The classification of *Weigeltisaurus* is uncertain and it has been variously placed in the subclass Lepidosauria order Rhynchocephalia[4], in the Lepidosauria order Weigeltisauridae[5] and in the subclass Euryapsida order Araeoscelidia[6]. Although similar to the Hetton reptile, it is larger and differs in the proportions of the skeletal elements. The two other (fragmentary) German specimens, pending a full description, have been tentatively referred to the genus *Weigeltisaurus*[7] but correspond in size and form more closely to the Hetton specimen. These three small reptiles are probably co-specific but their relationship to *Weigeltisaurus* is uncertain. There are about 15 species of the modern gliding lizard *Draco*, varying in size and weight from 5–6 g to 30 g in *Draco maximus*, the largest species[3]. By analogy, it is possible that the size range of the Permian gliding reptiles is attributable to different species of the genus *Weigeltisaurus*. Alternatively, they may represent sexual dimorphism within one species. Another gliding reptile, *Daedalosaurus madagascariensis*, has been described from the Upper Permian of Madagascar[8]. This poorly preserved lepidosaurian has 21 pairs of elongated ribs and shows both anatomical differences and similarities to the European reptiles. More research is required to clarify the classification and relationships of all these reptiles.

Similarly adapted reptiles include the Upper Triassic Kuhneosauridae, which possess 10–11 pairs of elongated ribs[3,9] and species of the aforementioned agamid lizard *Draco*, with 5–7 pairs[3]. The elongated ribs and other similarities of functional morphology can be related to the specialised ecology of these reptiles exemplified by *Draco*, an insectivorous form living aboreally in the rain forests of South East Asia[3]. Similar palaeoenvironmental conditions along the Zechstein Sea coasts are indicated by the diverse flora in the Marl Slate[1,10] and Kupferschiefer[11] which has also yielded insects[12,13].

I interpret the recurring adaptations in the Permian, Triassic and present-day gliding reptiles as the products of repetitive convergent evolution enabling exploitation of a highly specialised ecological niche.

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Negatively correlated cross-resistance to a synthetic pyrethroid in organophosphorus-resistant *Tetranychus urticae*

R. B. Chapman & D. R. Penman

Department of Entomology, Lincoln College, Canterbury,
New Zealand

Spider mites are serious pests of food and fibre crops and often cause considerable reductions in yields. Control is usually by regularly spraying chemicals, but frequently this approach has led to the development of resistance to these chemicals and ultimately to reduced levels of control[1–3]. A change to another chemical is then necessary. Most species of tetranychid mites of agricultural significance can develop resistance to many organophosphorus insecticides, some organochlorine and carbamate insecticides and various other compounds. Cross-resistance, where resistance to one compound confers resistance to other compounds of the same group, and multiple resistance, where resistance has developed to compounds from a number of structurally different groups, are also known to occur. Occasionally the development of resistance to one compound will have the reverse effect by making a population more susceptible to a newly introduced compound. This effect, termed negatively correlated cross-resistance, could have significant practical advantage in restoring some degree of susceptibility in a pest population. In this letter we describe an example of this phenomenon in spider mites in which resistance to organophosphates was linked with increased susceptibility to synthetic pyrethroids.

A strain of two-spotted spider mite, *Tetranychus urticae* Koch, resistant to an organophosphorus (OP) insecticide, was

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Table 1 Relative toxicities of azinphos-methyl and fenvalerate to strains of *T. urticae*

Population	Azinphos-methyl $LC_{50}(g\,l^{-1})$	Azinphos-methyl 95% Cl for LC_{50}	Azinphos-methyl Slope	Fenvalerate $LC_{50}(g\,l^{-1})$	Fenvalerate 95% Cl for LC_{50}	Fenvalerate Slope
A	0.564	0.482–0.661	4.71	0.021	0.016–0.028	2.05
B	0.432	0.349–0.533	3.41	0.018	0.015–0.022	4.16
C	0.418	0.340–0.514	3.22	0.019	0.014–0.025	2.43
D	0.408	0.330–0.502	3.15	0.018	0.015–0.036	4.16
E	0.273	0.220–0.340	3.55	0.031	0.023–0.043	2.19
F	0.213	0.170–0.265	2.68	0.022	0.015–0.032	1.93
G	0.108	0.086–0.137	4.82	0.027	0.019–0.040	1.82
H	0.068	0.050–0.093	1.73	0.039	0.031–0.048	3.00
I	0.063	0.034–0.120	2.42	0.041	0.029–0.058	1.97
J	0.058	0.045–0.073	2.43	0.156	0.113–0.214	1.73
K	0.051	0.040–0.065	2.82	0.187	0.015–0.232	3.82
L	0.023	0.018–0.030	2.54	0.079	0.052–0.121	1.53

Toxicity tests were performed by attaching 30 adult female mites to glass microscope slides with double-sided adhesive tape and immersing for 5 s in serially diluted solutions (5–7 concentrations) of the candidate insecticide. Solutions were prepared by dissolving technical grade samples (95%–99% pure) of fenvalerate and azinphos-methyl in an acetone: water (1:1) solvent. To determine solvent-induced mortality, mites were also immersed in solvent alone. Control mortality, which did not exceed 10%, was used to correct concentration-mortality data by Abbott's formula[8]. All dipping was performed at 25 ± 1 °C and 70–80% relative humidity for 24 h.

observed to be more susceptible to a synthetic pyrethroid (SP) insecticide than was a strain which had no history of exposure to insecticides. To confirm this observation populations of two-spotted spider mites from apple orchards in the Canterbury Province, New Zealand, were sampled for further laboratory tests. In these orchards the OP insecticide azinphos-methyl had been sprayed 5–10 times each year for many years to control lepidopterous pests. Spider mites in these orchards have become resistant to this insecticide. As a comparison, spider mite populations from home gardens where no sprays have been applied were also sampled for testing. The toxicities of azinphos-methyl and the SP insecticide fenvalerate were then determined by a standard laboratory slide dip technique[4]. Mortality was assessed after 24 h and the concentration–mortality data were subjected to probit analysis[5] to determine median lethal concentration (LC_{50}) values. Results for both insecticides are presented in Table 1 and Fig. 1.

Negatively correlated cross-resistance is clearly demonstrated, increasing azinphos-methyl resistance confering an increase in fenvalerate susceptibility. Populations of two-spotted spider mite with no history of exposure to insecticides were relatively more tolerant of fenvalerate than were field populations subjected to azinphos-methyl selection pressure. This phenomenon is uncommon among spider mites and has only been reported where resistance to the OP insecticide oxydemetonmethyl enhanced susceptibility to the formamidine compound, chlordimeform[6] and the carbamate insecticide formetanate[7]. Our results therefore suggest that by using fenvalerate a superior level of control could be achieved with azinphos-methyl resistant populations than with less resistant populations. The inclusion of fenvalerate in spray treatments in orchards where azinphos-methyl resistant mites abound may control these mites and eliminate the need for a specific acaricide. This would conserve the limited resource of effective chemicals.

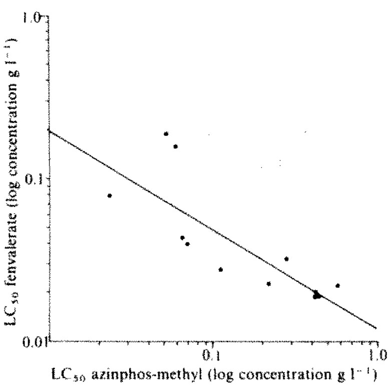

Fig. 1 Relationship between LC_{50} values of azinphos-methyl and fenvalerate for strains of two-spotted spider mite ($y = 0.011x^{-0.623}$; $r = 0.804$; $P > 0.01$).

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A virus which lyses the marine nanoflagellate *Micromonas pusilla*

J. A. Mayer* & F. J. R. Taylor†

Departments of Botany and Oceanography, University of British Columbia, Vancouver, British Columbia, Canada V6T 1W5

Observations of virus-like particles (VLPs) are now commonplace in a wide range of cell types. In most cases these observations represent incidental findings reported during a general ultrastructural investigation. Frequently in such instances it is impossible to establish the viral identity of the observed particles because the sample material containing the inclusions is no longer available and/or the data provided are inadequate. In particular, the number of established viral infections of algae[1,2] or protozoa[3] is small, and those amenable to continued study even smaller. We now report the isolation and ultrastructural identification of a lytic virus infecting the naked, marine phytoflagellate *Micromonas pusilla* (Butcher) Manton et Parke and describe its unusual mode of entry into the host.

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* Present address: Research School of Biological Sciences, The Australian National University, PO Box 475, Canberra City, ACT 2601 Australia.
† To whom reprint requests should be addressed.

Micromonas pusilla is an extremely small (1.5 to 3.0 μm) photosynthetic flagellate which occurs widely in both coastal and oceanic waters and is occasionally present in large numbers (10^3–10^5 cells l^{-1})[4]. It is therefore a frequently overlooked but important member of the marine nanoplankton. *Micromonas pusilla* is usually considered to be an anomalous member of the Prasinophyceae[5,6]. Although plant-like in that it is photosynthetic, it lacks a cell wall.

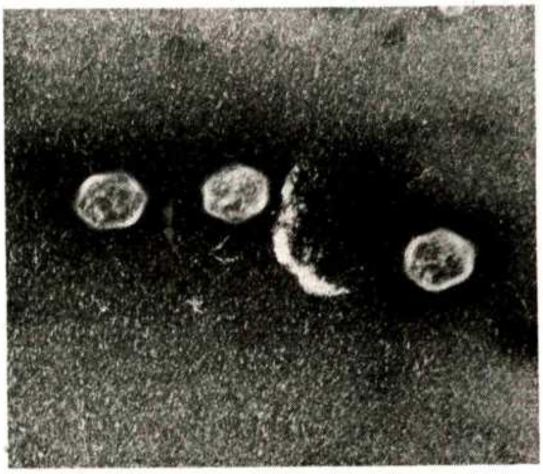

Fig. 1 Transmission electron micrograph of negatively stained MPV particles in clarified lysate from a culture of *M. pusilla*. ×76,500.

During a transmission electron microscope (TEM) study of natural communities of nanoplankton in the Strait of Georgia, British Columbia, in April 1975, polyhedral VLPs were observed within *Micromonas* cells from an enrichment culture of wild, mixed British Columbia nanoplankton. It was noted that *M. pusilla* disappeared rapidly from the enrichment culture and that medium from such a contaminated culture, after filtration through a 0.22-μm Millipore membrane filter, could cause lysis of a previously healthy unialgal culture. This infective filtrate caused the destruction of cultures of four unialgal strains of *M. pusilla* originating from both Atlantic and Pacific Oceans and maintained in the Northeast Pacific Culture Collection in the Department of Oceanography at the University of British Columbia.

Medium from lysed *M. pusilla* cultures was spun at 10,000*g* for 30 min to remove cell debris and negatively stained with 2% uranyl acetate. TEM examination of the clarified lysate revealed particles with hexagonal or pentagonal outlines measuring 130–135 nm in diameter (Fig. 1). No tail-like structures or other external appendages were observed.

The clarified lysate was inoculated into actively growing cultures of *M. pusilla* (whose cells, when examined with TEM,

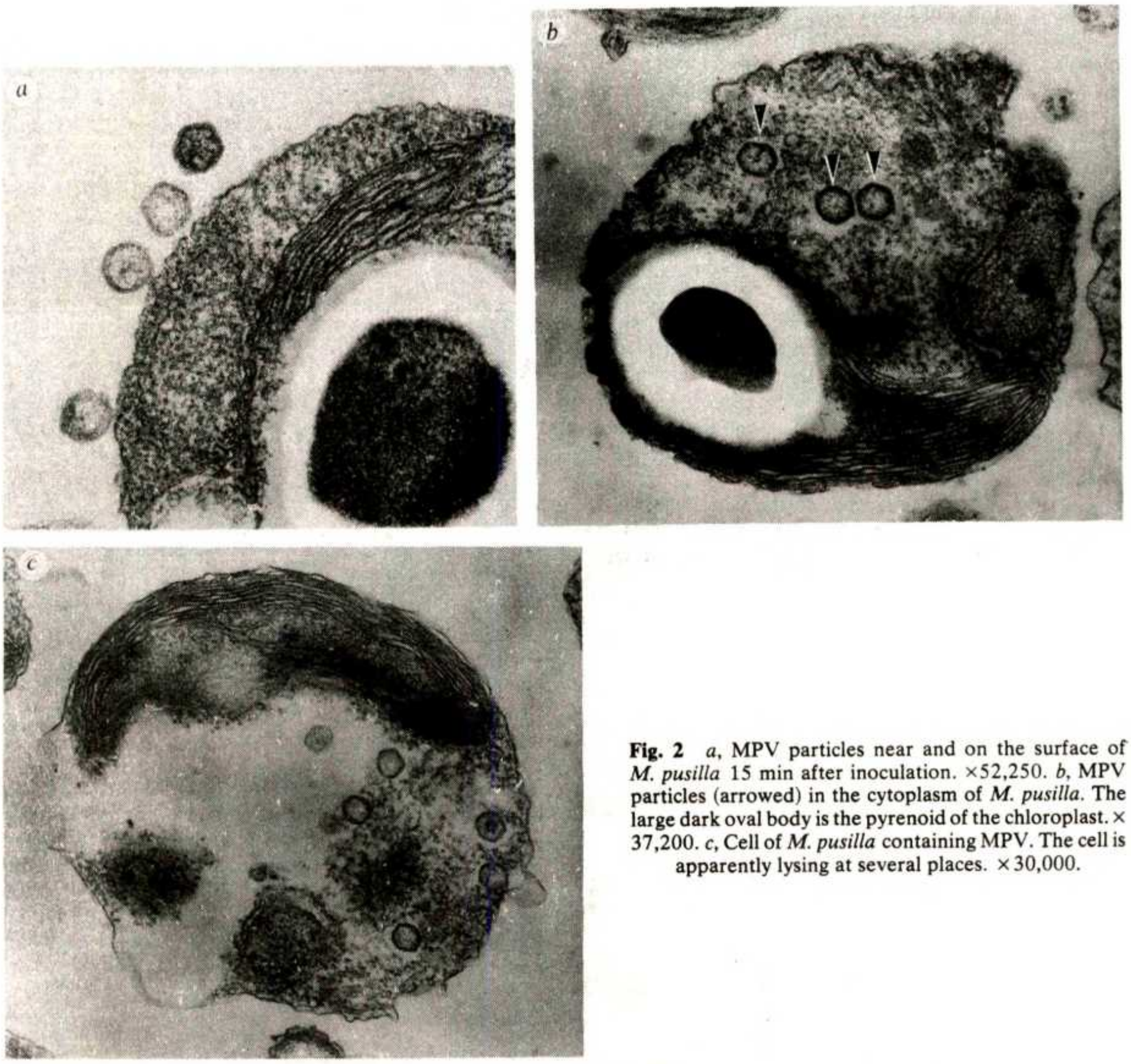

Fig. 2 *a*, MPV particles near and on the surface of *M. pusilla* 15 min after inoculation. ×52,250. *b*, MPV particles (arrowed) in the cytoplasm of *M. pusilla*. The large dark oval body is the pyrenoid of the chloroplast. × 37,200. *c*, Cell of *M. pusilla* containing MPV. The cell is apparently lysing at several places. ×30,000.

did not exhibit particles). Ultrastructural examination of these cultures indicated a predictable series of events. (1) Within 15 min polyhedral particles adhere to host cell surfaces (Fig. 2*a*). This localised attachment is followed by the fusion of adjacent host and particle surfaces, establishing confluence between the particle core and host cytoplasm. Particle entry by host phagocytosis (viropexis) was never observed. (2) Early stages of interaction described above are followed by an 'eclipse period' of approximately 3 h. Cells examined during this interval demonstrate no ultrastructural peculiarities. (3) Progeny particles appear within the cytoplasm (Fig. 2*b*). Their appearance is associated with the production of large amounts of scattered cytoplasmic fibrils (~5–8 nm in diameter) and clusters of small, membrane-bound vesicles never present in 'healthy' cells. Particles were never seen within the nucleus or associated with specific cell organelles. The *in situ*, sectional views of the particles are similar in shape and size to those observed in negatively stained preparations of the isolated particles. (4) Progeny particles are released by localised rupture of the cell membrane, often seen at several locations in the same cell, the cell contents including the particles being released into the medium (Fig. 2*c*).

Regularly shaped, polyhedral, intracellular structures, first observed in a naturally occurring population of *M. pusilla*, have been isolated and described. We conclude that the following evidence is sufficient to establish the viral nature of these entities. First, the particles can be introduced into and cause lysis of four geographical isolates of *M. pusilla*. Second, the particles pass through a 0.22-μm filter. This filtrate causes lysis as described above. Third, the morphology of these particles is similar to that of known viruses, and finally, the location of the particles relative to the time from introduction until cell lysis is consistent with that of a viral infection.

The particles have been tested with 46 planktonic algal isolates representing 35 species from 5 classes. Lysis occurred only in the four isolates of *M. pusilla*. The virus has been designated '*Micromonas pusilla* virus' (MPV).

This is the first case of controlled viral destruction of a marine phytoplankton organism known to us. Freshwater cyanophyte phytoplankters have been reported to be destroyed by 'cyanophages'[7]. The infection of these walled prokaryotic hosts resembles bacteriophage activity and is quite different from that of the small naked eukaryotic cells described here. The mode of entry of MPV seems unlike any virus previously described. MPV also differs from other algal viruses in that penetration through a cell wall is not required. As the virus is known to occur in natural seawater, at least in British Columbia and neighbouring Washington state[8], the ecological effect of MPV virus on the population dynamics of *M. pusilla* should be investigated.

As characterisation of MPV has not been completed, the group of viruses to which it belongs is unknown. On the basis of its large size, general morphology and apparent cytoplasmic site of assembly, MPV resembles a large number of viruses which have been referred to collectively as icosahedral cytoplasmic deoxyriboviruses[9,10] or the Iridoviridae[11]. This group of viruses is characterised in part by the presence of a DNA genome and by the iridescent appearance of crystals formed within their multicellular hosts or pellets of centrifuged virus. The definitive relationship of this newly identified viral pathogen with known virus groups can be determined only after information on its biochemical and biophysical properties becomes available.

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Rapid deposition of subcutaneous areolar tissue in response to sexual stimulation in mice

F. H. Bronson

The Institute of Reproductive Biology, Department of Zoology, University of Texas, Austin, Texas 78712

Many of the reproductive processes of house mice are regulated exteroceptively by social cues[1]. Puberty in a female mouse, for example, is a labile event that can be accelerated markedly if the young female is housed with an adult male[2]. The relevant male stimuli in this case are a combination of pheromonal and tactile cues[3–5]. The female's responses to these cues include an immediate, sequential release of gonadotropic and ovarian hormones[6], which in turn evoke a series of predictable changes in most of the female's reproductive tissues and culminate in the pubertal ovulation. I report here an unexpected consequence of exposing a young female mouse to a male, namely an intense response of a non-reproductive tissue. Specifically, young female mice experience a rapid thickening of their subcutaneous areolar tissue when they are exposed to males. The proximal cause of this response seems to be largely, but not exclusively, an enhanced secretion of ovarian oestrogen.

The phenomenon is illustrated in Fig. 1. In these preliminary experiments, CF1 female mice were weaned at 21–24 d and immediately housed in a room free of males. When they weighed 16–17 g, the females were either regrouped in the same room or paired individually with adult males in another room, a standard procedure for studying the socially induced acceleration of puberty in mice[2,6]. All females were killed after 3 d of such housing. Samples of skin (1 cm^2) were removed from the back of each female, fixed in formalin and stained with haematoxylin and eosin. The examples presented in Fig. 1 are typical of the results. The subcutaneous tissue in all 10 females exposed to males was 5–20 times thicker than that of any of the 10 control females. This thickening seemed to occur under the skin on all parts of the body but was studied in detail only on the back.

Many types of connective tissue are known to be sensitive to steroids of ovarian and/or adrenal origin[7,8], although the areolar tissue under the skin seems to have been routinely ignored in such studies[9]. A second experiment therefore examined the possible role of the ovary as a mediator of the socially enhanced deposition of areolar tissue. A group of 16 females (15–16 g) was ovariectomised or subjected to sham operations. Following 3 d of recovery, these females were either paired with males or housed in groups in a room free of males. Dorsal skin samples

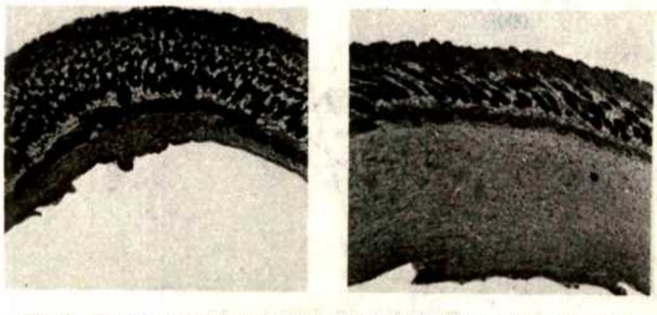

Fig. 1 Sections of skin taken from the backs of young female mice after the animals had been kept in groups in a male-free room (left) or individually paired with a male for 3 d (right). The thicker layer of subcutaneous connective tissue underlying the skin in the section on the right is obvious. The difference in thickness of the cutaneous layers themselves is not related to any experimental variable; such variation between individuals is normal for this stock of mice.

Table 1 Changes in thickness of subcutaneous areolar tissue and mean uterine weight in young female mice following ovariectomy

	Areolar tissue (μm)		Uterine weight (mg)	
	Ovx	Sham	Ovx	Sham
Male-exposed	142±7	1,602±111	10±1	115±8
Grouped	99±2	109±8	11±1	23±3

The female mice were ovariectomised (Ovx) or sham operated and then either exposed to males or held in groups for 4 d ($n = 4$ in each case). The results are the mean±s.e.

were collected 4 d later and sectioned on a cryostat; the thickness of the areolar tissue was estimated in repeated samples with a micrometer eyepiece. The results in Table 1 show an average 15-fold increase in the thickness of this tissue in those sham-operated females exposed to males. This response to a male was largely but not entirely eliminated by ovariectomy. Table 1 also records some basic differences between subcutaneous connective tissue and the uterus with regard to their relative responsiveness to ovariectomy and male stimulation. Exposing a young female mouse to a male results in a sequential release of luteinising hormone from the pituitary and oestradiol from the ovary[6]. The uterus gains weight rapidly in response to the enhanced secretion of oestradiol and, as shown in Table 1, ovariectomy completely abolishes such male-induced growth of the uterus but is not completely effective in blocking the deposition of subcutaneous areolar tissue in response to male stimulation. Furthermore, the uterus is markedly and significantly ($P < 0.01$) smaller 4 d after ovariectomy whereas the increase in the thickness of subcutaneous areolar tissue at this time is not significantly depressed by ovariectomy.

The results of a series of pilot studies, each of which involved the injection of steroid or pituitary hormones, suggested that the female mouse's subcutaneous connective tissue is strongly responsive to oestradiol but not to several other hormones, including progesterone and ACTH. Figure 2 shows a dose–response curve of areolar tissue thickness in young females implanted with various doses of oestradiol. These females were ovariectomised at 15–16 g and allowed 3 d to recover, after which they were implanted with hormone. Oestradiol implants were made by mixing 17β-oestradiol with Silastic adhesive (Type A, No. 891, Dow Corning). The resulting suspension was packed in a 10-mm Silastic capsule (i.d. 0.04 mm; o.d. 0.085 mm, Dow Corning). A suspension of 0.1 mg oestradiol per ml resulted in a significant thickening of the areolar tissue ($P < 0.01$) although the uterus was unresponsive to this dose of oestradiol. Both the uterus and areolar tissue responded strongly to doses above 0.1 mg ml^{-1}. With 10 mg ml^{-1} there was slightly more deposition of areolar tissue than occurred after 4 d of male exposure in an intact female (see Fig. 2 and Table 1).

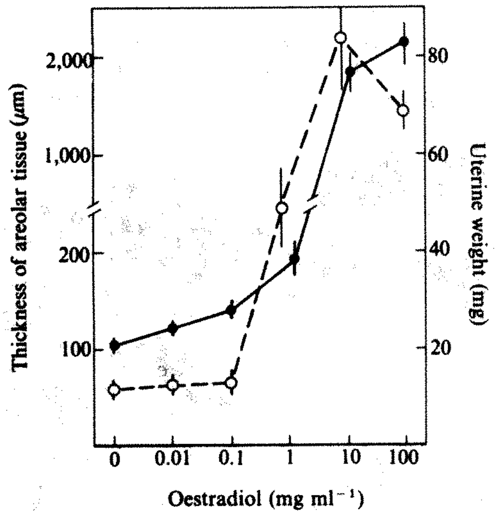

Fig. 2 Mean thickness of subcutaneous areolar tissue (μm±s.e.) (●) and mean uterine weight (mg±s.e.) (○) in young female mice that were ovariectomised and implanted with Silastic capsules contaning oestradiol.

What might be the adaptive function of the deposition of this layer of subcutaneous tissue in response to a male? This layer of connective tissue does not anchor the skin to the underlying musculature in the mouse, as it does in some other mammals[10]. Despite the total absence of fat cells in this tissue when under the skin of the back (Fig. 1), it is impregnated with fat cells in the area of the mammary glands, but the preponderance of such cells does not seem to change at puberty. Thus, one would not expect the pubertal increase in this layer of connective tissue to be important for post-pubertal steroid metabolism or thermal insulation. One possibility is that, as this layer of connective tissue envelops the mammary glands ventrally, its thickening in response to oestrogen could be a provision for the support of these glands when they enlarge during late pregnancy, and could subsequently serve to anchor them firmly to the skin during lactation. Whatever the function for this tissue response, however, the present results do show that in the mouse this tissue is an important target of oestrogen action, and is perhaps even more sensitive to oestrogen than the organ best recognised as a target for this steroid, the uterus.

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Resistance of mice suppressed for IgM production to *Babesia microti* infection

Yvonne J. Rosenberg & Charles B. Evans

Laboratory of Microbial Immunity, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20205

The immunological mechanisms responsible for overcoming infections with *Babesia*, an intra-erythrocytic protozoan, are not fully understood. Although high titres of specific anti-babesial antibodies have been observed in several species of animals[1], and protection has been obtained by transfer of large volumes of recovery serum[2], the role of antibody in the immune response to an infection is uncertain. The present study investigates the nature of B-cell participation during *Babesia microti* infections by observing the course of the disease in mice in which IgM production has been suppressed from birth and which contain no B cells. The results show that, in contrast to control mice, which develop and subsequently clear circulating parasitaemias, suppressed mice show an unexpected resistance to infection as reflected by a virtual absence of parasites in the peripheral circulation.

Table 1 Comparison of parasitaemias in IgM-suppressed and control mice following infection and challenge with *B. microti* (expt 1)

Mice	Days after initial infection										
	5	8	11	13	15	17	19	22	24	60*	67
IgM Suppressed	0	0	0	0		0	<0.2		9.6	D†	D
	0	0	0	0		0	<0.2		<0.2	0	0
	0	0	0	0		0	0		0	0	17.6
	0	0	0	0		0	0		0	0	<0.2
	0	0	0	0		0	0		0	0	0
	0	0	0	0		D	D		D	D	D
	0	0	0	0		0	0		<0.2	0	0
	0	0	0	0		0	0		D	D	D
Anti-ferritin antibody treated	0	<0.2	5.8	5.4	0.2	3.4	1.0	<0.2	0	<0.2	0
	0	<0.2	2.8	4.6	1.2	<0.2	0.6	<0.2	0	0	0
	<0.2	<0.2	22.4	24.4	8.4	3.4	4.8	0.8	<0.2	<0.2	0
	0	<0.2	5.6	0	ND	ND	<0.2	<0.2	0	0	0
	0	<0.2	9.2	11.6	8.0	3.6	0.8	<0.2	<0.2	0	0
Untreated normal controls	0	3.6	26.0	9.6	4.6	<0.2	1.2	<0.2	0	0	0
	0	4.0	11.6	12.0	10.0	9.0	4.0	0	<0.2	0	0
	0	8.6	41.6	14.2	9.0	4.2	4.8	<0.2	<0.2	0	0
	0	3.8	18.0	20.8	10.2	6.8	5.4	<0.2	<0.2	0	0
	0	10.2	17.0	6.2	4.4	0.8	1.8	<0.2	0	0	0
	<0.2	4.6	11.2	12.4	6.8	3.2	5.4	<0.2	<0.2	<0.2	0
	0	0.4	12.0	6.2	6.0	1.8	3.2	<0.2	0	0	<0.2

Three-month-old female B6CF$_1$ mice injected from birth with either GαMIgM (group 1) or goat anti-ferritin antibody (group 2), or untreated (group 3), were infected i.p. with 10^6 *B. microti* and per cent parasitaemias for each mouse determined by Giemsa stain. Suppressed mice were still negative for circulating IgM when tested by Ouchterlony analysis 29 days after infection. Removal of suppression at day 30 did not result in the appearance of parasites in peripheral smears 7 or 14 days later. On day 37, blood samples from the five remaining suppressed mice were subinoculated into B6CF$_1$ normal mice. Three of the recipients had low parasitaemias at day 12 after sub-inoculation.

*Homologous immunity was tested on day 53 by infection of all groups with 10^6 *B. microti* and smeared on day 7 (D60) and day 14 (D67) thereafter. Six months after challenge, groups 1 and 2 received 10^6 *P. yoelii* i.p. 90% Of mice died within 10 days. ND, not done; D, died.

†Died day 29.

The following three age-matched groups of (C57BL/6 × BALB/c)F$_1$ (B6CF$_1$) and BALB/c × C57BL/6)F$_1$ (CB6F$_1$) mice, each containing 4–10 animals, were intraperitoneally (i.p.) infected with 10^6 *B. microti* (Kings 67 strain): (1) mice suppressed from birth with a globulin fraction of goat anti-mouse IgM (GαMIgM)[3], and which showed no circulating IgM at the time of infection (suppressed group); (2) mice which received a globulin fraction of goat anti-ferritin antibody in a similar injection regime, to control for any effects of repeated antibody injections (antibody controls), and (3) normal, untreated mice (normal controls). Parasitaemias, determined by Giemsa stain of thin smears from tail blood, were first examined at various times after primary infection with *Babesia*, again in the succeeding interval during which the IgM suppression was discontinued, and finally, following challenge with the same parasite. Thereafter, immunity to a heterologous parasite was tested by infection with 10^6 lethal malaria (17 × *Plasmodium yoelii*). The data from two such experiments, which compare the per cent parasitised erythrocytes observed in the periphery of suppressed versus both control groups, are presented in Tables 1 and 2.

Results from the first experiment, which used 3-month-old B6CF$_1$ female mice throughout, are shown in Table 1. As can be seen, in contrast to the rise and fall in parasitaemias observed in either control group, the suppressed group showed a virtual absence of parasitised red blood cells (RBC) in the circulation (except for one mouse which had 9.6% of cells infected). Although this apparent lack of parasites continued when mice were released from suppression, low numbers must have been present, as three out of five of the remaining suppressed mice were positive on sub-inoculation 37 days after infection. Despite the fact that parasites were rarely seen in the suppressed mice following primary infection, these animals nevertheless seemed to be as well protected as control mice against homologous challenge 7 weeks later. In addition, both the suppressed and control groups exhibited a lack of heterologous immunity after infection with lethal *P. yoelii* several months after primary infection with *B. microti*. These findings are similar to those of Cox using non-lethal *P. yoelii*[4].

It is possible that the coating of parasitised cells in recipient mice by GαMIgM either directly, or indirectly through passively transferred IgM on infected cells, could have resulted in blocking of infection by parasites or rapid clearance of infected cells by macrophages in the suppressed mice. To test for such effects, the parasitised cells used in the second experiment were washed three times in saline before infection and, in addition, a portion of these washed cells was incubated in GαMIgM serum for 30 min at 4 °C before injection into normal mice, to examine whether cells deliberately coated with Ig were rendered non-infectious. In this experiment, 2-month-old B6CF$_1$ mice (suppressed and antibody control groups) and CB6F$_1$ mice (normal group) of both sexes were used, and four normal 4-month-old female B6CF$_1$ mice also served as controls.

The results, presented in Table 2, are similar to those obtained in the first experiment in that (1) suppressed mice showed almost no sign of parasites compared with the controls, (2) removal of suppression had no effect on the levels of parasitaemia, and (3) the degree of protection on challenge was similar in each group. The differences in parasitaemia between the antibody and normal control groups which occurred in the first experiment were not seen in experiment 2. The lower peak of parasitaemias seen during the second experiment, however, probably reflects the fact that these mice were 4 weeks younger than those used in experiment 1, as the 4-month-old normal control mice developed much higher parasitaemias with the same inoculating dose. These results also suggested that at least in the younger mice, a sex-related susceptibility to infection with *B. microti* exists, as male mice always had higher parasitaemias than females. Furthermore, the preincubation of the parasitised cells in GαMIgM did not affect the ability of the parasite to infect and multiply, but rather resulted in consistently higher parasitaemias (Table 2). As in experiment 1, all mice were equally protected following reinfection with *B. microti*, although low parasitaemias in recipients of sub-inoculated blood from all groups indicated that low levels of infection persisted after homologous challenge. As an additional control for the effects of GαMIgM injections, an experiment was done in which normal adult

B6CF$_1$ mice were given the antisera for 8 days before and 22 days after infection with 10^6 *B. microti*. These mice developed parasitaemias similar to those seen in normal control mice.

Previous studies have suggested that an intact complement system is essential for the development of Babesial parasitaemias[5,6], and more recently, Chapman and Ward[7] demonstrated that factors of the alternate pathway as well as the third (C3) and fifth (C5) components from human or rat serum are required for *Babesia rhodaini* penetration of human red cells *in vitro*. As it was possible that the absence of parasitaemia in IgM-suppressed mice reflected a deficiency in the required complement components, serum samples from these animals were tested for C3 activity by immune adherence[8]. This assay uses the fact that human erythrocytes which have a receptor for the third component of complement (C3b) will adhere to sensitised sheep RBC preincubated in serum containing C3 components (as well as C1, C4 and C2). The results (not shown) indicated that the C3 titres in three suppressed mice were in the same range as those in the three control mice and makes it unlikely that a C3 deficiency could explain the above results.

Based on the present data, the simplest explanation for the apparent lack of susceptibility to infection of suppressed mice is that, to invade mature RBC, *Babesia* parasites require IgM antibody; the absence of the capacity to make IgM antibody thus confers resistance. Although the specificity of such an antibody is not known, an interesting possibility is that an autoantibody with specificity for membrane determinants on parasitised or normal erythrocytes is required for invasion. Antibodies reactive with antigens on enzyme-treated isologous RBC have been demonstrated during protozoal infections[9,10] and are also elicited following infection of normal mice with *B. microti* (not shown). Perhaps, therefore, red cell antigen–antibody complexes in association with complement facilitate penetration of the parasite into erythrocytes by alteration of the red cell membrane.

However, several other interpretations of the data are possible. For example, because only peripheral smears were examined, parasites sequestered in specific organs would not have been scored. In addition, the observed resistance might be explained by increases in the activity of natural killer (NK) cells[11,12] or macrophages in the suppressed mice. A protective role for such cells, however, is not compatible with the studies of Weinbaum *et al.*[13] which demonstrate the ability of another non-lethal parasite, 17X *P. yoelii*, to infect and eventually kill similarly suppressed mice. It is not known whether the mechanisms preventing a primary parasitaemia are the same as those which result in protection on challenge. Thus, although IgM suppression could explain the lack of parasitaemias following the initial infection, it is possible that T cells primed by the few parasites present during that time are effective in protecting mice after suppression is discontinued[14]. For this reason, the present observations probably reflect the existence of several different mechanisms responsible for the protection observed in the IgM-suppressed mice, some of which may be similar to those resulting in the phenomenon of age resistance in young cattle[15].

Table 2 Comparison of parasitaemias in IgM-suppressed and control mice following infection and challenge with *B. microti* (expt 2)

Mice	Sex	No. per group	7	9	12	13	14	19	35	111*	121
IgM-suppressed B6CF$_1$	M	6	0	0	0	0	0	0.007 (0–0.02)	0.003 (0–0.02)	0	0
	F	5	0	0	0	0	0	0.08 (0–0.4)	0	0	0
Antibody control B6CF$_1$	M	4	0.015 (0–0.02)	1.9 (0–3.6)	9.2 (0.02–15.4)	11.1 (0–22.6)	5.4 (0–14.8)	0.17 (0.02–0.6)	0	0	0
	F	9	1.2 (0–5.4)	6.6 (0–17.4)	4.7 (0–11.6)	2.8 (0–12.2)	1.1 (0.2–5.6)	3.2 (0.2–0.204)	0	0	0
Normal control CB6F$_1$	M	4	0.3 (0–0.6)	4.1 (0–15.2)	9.2 (0.2–31)	4.3 (0.2–14.8)	2.7 (0.4–4.6)	7.3 (0.02–26.8)	0	0	0
	F	5	0.13 (0.02–0.6)	0.32 (0.02–0.8)	3.5 (0.02–11.4)	0.93 (0–2.4)	0.28 (0–0.8)	0.008 (0–0.02)	0.005 (0–0.02)	0	0
†Normal control CB6F$_1$	M	3	0.27 (0.02–0.6)	7.4 (4.8–10.4)	16.5 (9.6–23)	12.3 (20–27.6)	7.2 (2.6–14.4)	0.4 (0–0.6)	0	0	0
	F	3	0.02 (0.02)	2.7 (0.2–5.4)	4.1 (2.6–5.4)	0.28 (0.02–0.6)	0.74 (0.02–2)	0.07 (0.02–0.2)	0	0	0
‡Normal control B6CF$_1$	F	4	0.26 (0.02–0.4)	6.9 (3.8–10)	25.3 (14–46.8)	22.8 (17.2–30.8)	18.5 (11–21.8)	3.5 (0.6–7)	0	0.005 (0–0.2)	0.01 (0–0.4)

Male and female 2-month-old B6CF$_1$ mice (IgM-suppressed and antibody control groups) and CB6F$_1$ mice (normal controls) were infected with 10^6 *B. microti* i.p. and parasitaemias determined by Giemsa stain. Data are expressed as mean per cent parasitaemia in each group. The range for each group is given in parentheses. All parasitised RBC were washed three times in saline before injection.

* All groups were challenged on day 103 with 10^6 *B. microti*.

† Washed parasitised RBC were preincubated in GαMIgM for 30 min at 4 °C before injection.

‡ Four-month-old normal B6CF$_1$ controls.

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Cross-linking of lymphocytic surface immunoglobulin inhibits its production via a cyclic nucleotide mechanism

M. Glennie, F. K. Stevenson, G. T. Stevenson & M. Virji

Tenovus Research Laboratory, Southampton General Hospital,
Southampton, UK

When antibody to immunoglobulin (anti-Ig) reacts with Ig molecules on B-lymphocyte surfaces the Ig, tethered by the antibody, moves over the surface to form patches and finally a cap at one pole of the cell. Simultaneously, the Ig–antibody complexes are removed by pinocytosis at the patches and cap, until the surface is cleared of the target molecules[1,2]. In immunofluorescent studies, Sidman and Unanue[3] followed the reappearance of the surface Ig during subsequent culture of the cells. They observed that splenic lymphocytes from adult mice re-express surface Ig promptly, whereas those from neonatal animals re-express poorly or not at all. They suggested that this phenomenon might be related to the greater susceptibility of lymphocytes from immature animals to tolerisation by antigen. Such an interpretation was supported by a simultaneous study[4] showing that explanted mouse fetal lymphocytes were less able to re-express surface Ig after treatment by anti-Ig than were adult lymphocytes. However, Ault and Unanue[5] later reported in man a similar dichotomy between blood and nodal lymphocytes, the former re-expressing surface Ig more poorly. We report here studies on guinea pig leukaemic lymphocytes, which have also shown delayed re-expression of surface Ig after clearing by antibody. We present evidence that the delayed re-expression reflects a reduction in the normal delivery rate associated with turnover at the surface. The reduction follows a train of events initiated by cross-linking of the surface Ig and including an increase in intracellular cyclic AMP. Furthermore, the shutdown in delivery is apparently specific, inasmuch as delivery of two other surface molecules we have been able to quantify proceeds unimpeded. These observations suggest a basic mechanism expressed to varying extents by normal lymphoid populations to account for their differing rates of surface Ig regeneration[3–5].

The L_2C leukaemia arose spontaneously in a female strain 2 guinea pig and has since been maintained by passaging *in vivo* through syngeneic or near-syngeneic animals[6]. Cells for the present work were collected from the blood of animals in the terminal stages of the disease[7]. The cells show a surface chemistry typical of B lymphocytes[8]. The surface Ig is of class IgMλ with a density of approximately 50,000 molecules per cell and a half life on the surface of about 5 h (ref. 9).

The general experimental plan was to perturb the surface Ig of the L_2C cell in culture at 37 °C, by antibody or other means, to chill the cells, remove the perturbing agent and wash, and then to culture the cells again, sampling at intervals to determine the density of the Ig or other surface molecules. The anti-Ig used was raised in sheep against the C_λ determinants of guinea pig Ig, and was purified by affinity chromatography[10]. Antibodies to the histocompatibility antigens Ia(2,4) and GPLA(B1) were raised in guinea pigs: anti-Ia by injecting strain 13 animals with normal strain 2 splenic and nodal lymphocytes[11], and anti-GPLA by injecting Dunkin–Hartley animals (lacking GPLA(B1) on testing with an antiserum and matching strain 13 in Ia to the extent that testing was possible) with normal strain 13 lymphocytes. Estimations of the densities of L_2C surface molecules were carried out by allowing the cells to react at 4 °C with isotopically labelled antibody. The surface Ig was labelled directly by ^{125}I-Fab′γ prepared from purified anti-C_λ, the Ia and GPLA antigens indirectly by incubating first with IgG from the corresponding guinea pig antiserum and then, after an intermediate washing, with ^{125}I-labelled purified sheep anti-(guinea pig Fcγ). Antibody concentrations were on the plateaus of previously defined labelling curves. Labelling of controls by non-antibody Fab′γ or IgG was always negligible.

Figure 1*a* shows that the L_2C surface Ig is turning over with a half life of 5 h: this is demonstrated both by the rate of re-appearance of its Fab regions after their removal by papain (leaving the Fc regions attached to the membrane[12]), and by the

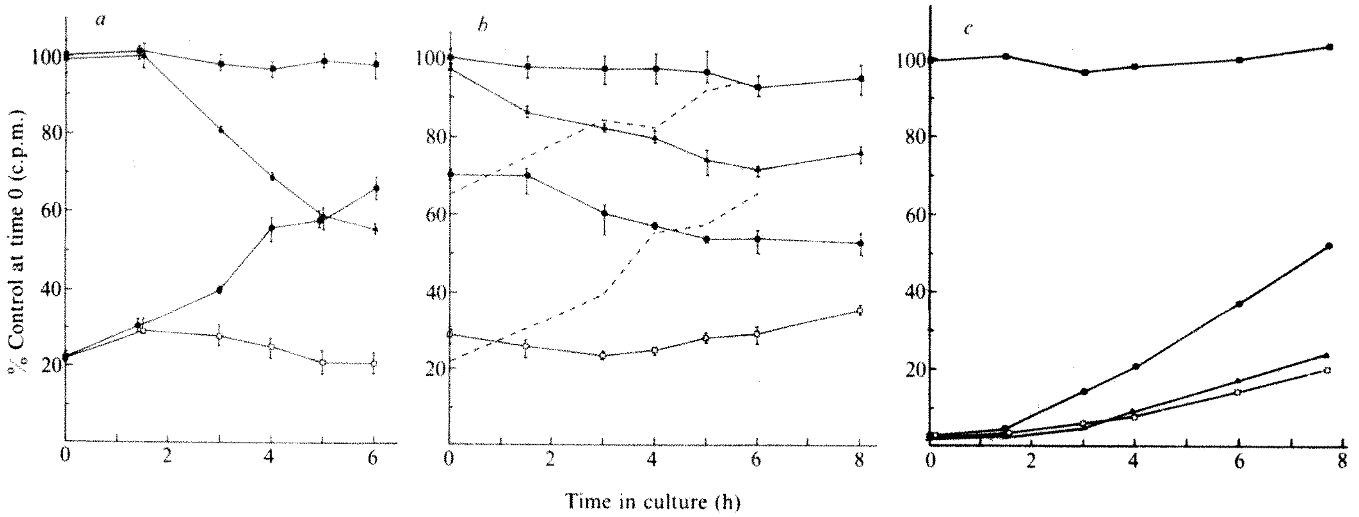

Fig. 1 Densities of surface Ig. After the treatments described below the cells were cultured with swirling at 37 °C in supplemented Eagle's medium (5 × 10^6 cells ml^{-1}), and were sampled at intervals to quantify the surface Ig by labelling with radioactive anti-Ig. The ranges shown are of triplicates. *a*, Normal turnover. ■, Control cells, treated as for the papain digestion but with the enzyme omitted; ●, treated with papain 0.1 mg ml^{-1} and dithiothreitol 0.1 mM for 30 min at 37 °C; ▲, treated as the controls, then cultured in the presence of 0.1 mM cycloheximide; □, treated with papain and dithiothreitol, then cultured in the presence of 0.1 mM cycloheximide. *b*, Reduced delivery following treatment with anti-Ig. Cells were exposed to sheep IgG at 50 μg ml^{-1} for 30 min at 37 °C before washing and culturing. The sheep IgG contained anti-Ig antibody at the following concentrations: ■, zero; ▲, 1 μg ml^{-1}; ●, 8 μg ml^{-1}; □, 50 μg ml^{-1}. - - - -, Parallel batches of cells treated with papain at 0.01 mg ml^{-1} (upper line) or 0.1 mg ml^{-1} (lower line), without exposure to antibody. *c*, Confirming reduced delivery due to anti-Ig. The sequential treatments were each of the same duration as in *a* and *b*, and were carried out with chilling and washing after each step. ■, Treated with normal IgG 200 μg ml^{-1}, followed by papain-free buffer; ●, treated with normal IgG 200 μg ml^{-1}, followed by papain 0.1 mg ml^{-1}; □, treated with anti-Ig 200 μg ml^{-1}, followed by papain-free buffer; ▲, treated with antibody 200 μg ml^{-1}, followed by papain 0.1 mg ml^{-1}.

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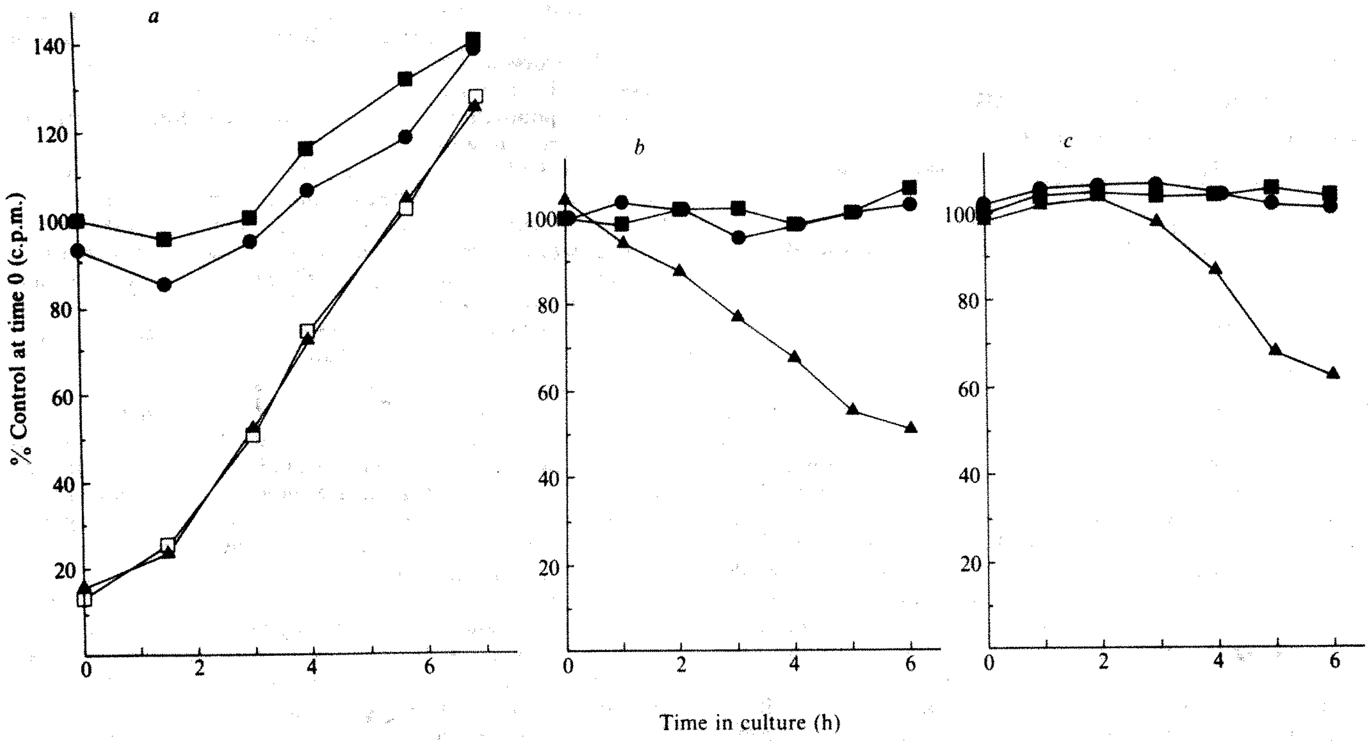

Fig. 2 Densities of surface GPLA and Ia. Ranges are omitted for clarity, but were similar to those shown in Fig. 1. *a*, Delivery of GPLA to the surface following treatments with anti-Ig and/or papain. ■, Treated with sheep normal IgG 200 μg ml^{-1}, then with papain-free buffer; ▲, treated with sheep normal IgG 200 μg ml^{-1}, then with papain 1.9 mg ml^{-1} for 30 min at 37 °C; ●, treated with anti-Ig 200 μg ml^{-1}, then with papain-free buffer; □, treated with anti-Ig, then with papain. *b*, *c*, Densities of surface GPLA and Ia following treatment with anti-Ig or cycloheximide. ■, Treated with sheep normal IgG 200 μg ml^{-1}; ●, treated with anti-Ig at 200 μg ml^{-1}; ▲, treated with sheep normal IgG, then cultured in the presence of 0.1 mM cycloheximide.

rate of disappearance of surface Ig after halting protein synthesis with cycloheximide. However, Fig. 1*b* indicates that after extensive clearing of the surface Ig by antibody at 37 °C there is no significant regeneration in subsequent culture until after about 4 h (lowermost solid line). Figure 1*b* demonstrates, moreover, that amounts of antibody insufficient to clear the surface completely by capping and pinocytosis (intermediate two solid lines) nonetheless induce a delay in delivery of new surface Ig. For about 6 h after the reaction with antibody the surface Ig continues to fall, implying that reduced delivery is not keeping pace with shedding. These rates of regeneration are in striking contrast to those shown in the same figure to occur after partial or complete clearing of the surface by papain.

Figure 1*c* shows that the action of antibody and papain sequentially yields the same slow regeneration as occurs after antibody alone. This rules out the possibility that the regeneration rate after antibody is normal but after papain spuriously high due to some stimulatory action of the enzyme. It also

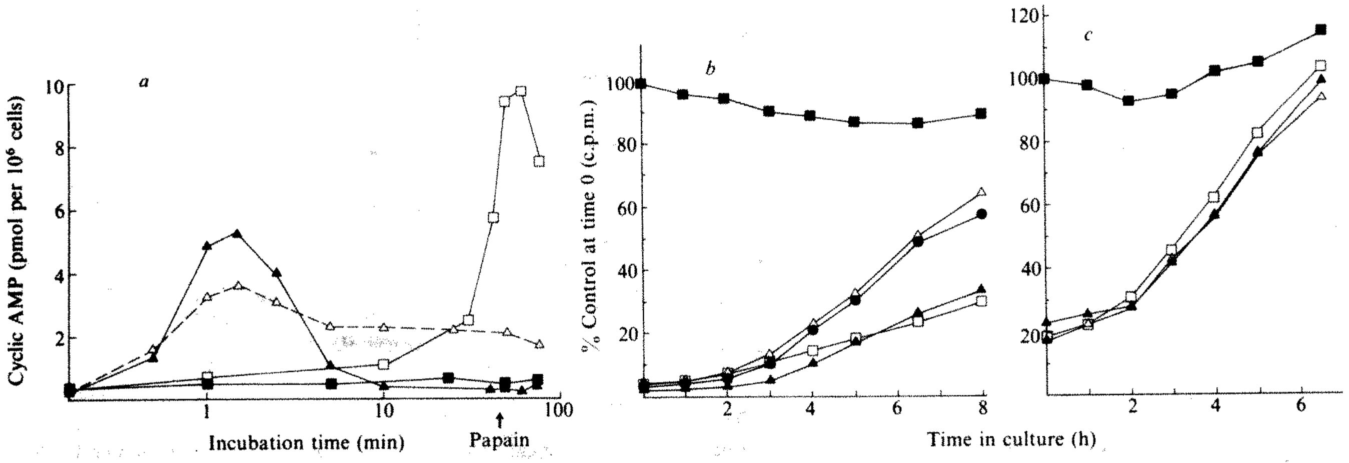

Fig. 3 Delivery of surface molecules correlated with cyclic AMP levels[14]. In *b* and *c* the ranges of triplicates were similar to those shown in Fig. 1. *a*, Levels of intracellular cyclic AMP following exposure for 45 min at 37 °C to: ■, sheep normal IgG, 200 μg ml^{-1}; ▲, anti-Ig, 200 μg ml^{-1}; △ isobutylmethylxanthine (Aldrich), 2 mM; □, cholera toxin (Sigma), 100 ng ml^{-1}. In the last three cases the cells were exposed to papain 0.1 mg ml^{-1} for 30 min at 37 °C immediately after exposure to the primary agent, so as to mimic conditions for the experiment depicted in *b*. The treatment with papain in fact made no difference to the levels of cyclic AMP attained, and viability (exclusion of Trypan blue) remained at >95% in all groups. *b*, Delivery of surface Ig after treatment for 45 min at 37° with: ▲, anti-Ig, 200 μg ml^{-1}; △, isobutylmethylxanthine, 2 mM; □, cholera toxin, 100 ng ml^{-1}. After these primary treatments the cells were treated with papain 0.1 mg ml^{-1} for 30 min at 37 °C to remove residual surface Ig, and finally washed and cultured to observe regeneration of the surface Ig. Two control groups were exposed to sheep normal IgG, 200 μg ml^{-1}, as the primary treatment: ●, followed by treatment with papain; ■, not followed by papain. *c*, Delivery of surface GPLA following treatment as in *b*, but with papain at 1.9 mg ml^{-1} to remove residual GPLA.

provides evidence against the possibility that delayed regeneration following antibody treatment is due to chronic clearance by persisting sheep antibody on the surface, because this antibody would itself have been susceptible to proteolysis by papain.

In experiments in which the cells were allowed to react with monovalent Fab′γ fragments of antibody, no effects were observed on the density of the surface Ig, whereas F(ab′γ)$_2$ fragments had the same effect as whole antibody. Thus, it seems that a necessary signal for delayed delivery is cross-linking of the surface Ig, with no apparent role for the antibody Fc region.

The experiments shown in Fig. 2 indicate that anti-Ig reacting with L$_2$C cells had no effect on the delivery of two non-Ig molecules, GPLA and Ia, to the cell surface. Figure 2*a* shows that the rate of regeneration of GPLA following its removal by papain is independent of the cell being exposed to anti-Ig. (The rising control values were often seen with this antigen, and might be related to the cell cycle[13].) A similar experiment could not be carried out for Ia, which could not be removed by any of a series of proteolytic enzymes tested. However, Fig. 2*b* and *c* show, respectively, that neither GPLA nor Ia underwent any diminution in density after the cells had been treated with anti-Ig, contrary to the result expected if delivery of these molecules were impeded. To confirm that both molecules are, in fact, turning over at the cell surface, the same graphs show their rates of fall-off when protein synthesis is halted by cycloheximide: the rates are apparently similar, but with a delay of about 2 h before the Ia starts to fall off, presumably reflecting the size of an intracellular pool.

There is some evidence of the pathway leading to suppression of Ig delivery. Previous work has shown that anti-Ig acting on the L$_2$C surface triggers a surge in cyclic AMP within the cell[14]. Protein kinases will thereby be activated, to phosphorylate other enzymes and possibly finally to yield reduced Ig delivery, among other effects. Such an interpretation is supported by the experiment shown in Fig. 3. Cholera toxin, binding to different receptors (ganglioside g_{M1}) on the surface[15], caused after a lag period a marked increase in cyclic AMP and then a reduced delivery of Ig but not of GPLA. The effect was strikingly similar to that given by anti-Ig. Another means of raising the cyclic AMP level, slowing its breakdown by the phosphodiesterase inhibitor isobutylmethylxanthine, had no detectable effect on delivery to the surface. Note, however, that the rise in cyclic AMP caused by this agent can be expected to occur throughout the cell rather than to be concentrated under the plasma membrane, a difference likely to give different sequelae[16].

Two lines of evidence suggest that antibody need only cross-link surface Ig to generate the signal which culminates in reduced Ig delivery. First, clearing by capping and pinocytosis is not necessary (Fig. 1*b*). Second, the surge in cyclic AMP is evident within 30 s (ref. 14), too short a time for any obvious interiorisation of antibody–Ig complexes[2]. Nevertheless, we cannot entirely rule out a need for at least minimal pinocytosis of the complexes.

We intend to apply the techniques used in this work to investigate other neoplastic lymphocytes, in particular, those of human chronic lymphocytic leukaemia. Clearly, caution must be exercised in extrapolating the behaviour of neoplastic cells to that of their normal counterparts. However, in the present case there is already evidence that the basic phenomenon is expressed to different degrees in normal cells[3–5,17]. Under such circumstances neoplastic cells, by virtue of their availability and homogeneity, offer considerable scope for elucidating mechanisms and perhaps for providing models of the normal stages of differentiation.

The perturbation of surface Ig in leukaemic lymphocytes by anti-Ig also has therapeutic implications. On these surface molecules there are idiotypic determinants, essentially unique to the clone of cells bearing them, and anti-Ig directed against these particular determinants may prove therapeutically useful[18].

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Human T lymphocyte clones reactive in primed lymphocyte typing and cytotoxicity

Fritz H. Bach, Hiroo Inouye, Jacquelyn A. Hank & Barbara J. Alter

Immunobiology Research Center, and Department of Medical Genetics and Surgery, University of Wisconsin, Madison, Wisconsin 53706

The human HLA-D region, the presumed evolutionary homologue of the H-2 I region in mouse and similar genetic regions in other species[1], codes for multiple antigenic determinants, a finding consistent with the existence of multiple loci[2,3]. One method developed to define D region encoded determinants is primed LD typing (PLT) wherein cells of one individual are primed *in vitro* in a mixed leukocyte culture (MLC) to allogeneic HLA-D encoded determinants associated with one haplotype[4,5]. The PLT cells give a rapid proliferative response when restimulated in secondary culture with either the priming cells or third party cells that carry the priming antigen(s). However, as in many cases a PLT cell is probably primed to several determinants, different restimulating cells from unrelated individuals will engender a range of responses varying from very high to very low, presumably depending on the number of determinants shared (or cross-reactive) between the priming and restimulating cells. Such results are difficult to interpret in terms of defining antigenic determinants on the cells of various individuals. Fathman and Hengartner[6] have circumvented this problem in mouse by cloning murine T lymphocytes from multiply restimulated PLT cells; the progeny of any one clone give highly specific proliferative responses. We report here the use of the supernatant of phytohaemagglutinin (PHA)-stimulated mixtures of human allogeneic cells[7,8] (designated T-cell growth factor or TCGF) to grow 'clones' of human T lymphocytes following alloactivation in MLC. Clones that give PLT-type responses do so in a highly antigen-specific manner. Following cloning, cells in many cases have undergone 25 or more divisions, thus yielding in excess of 1×10^8 progeny.

All cells to be cloned were alloactivated in primary MLC and on day 4 the blast cell fraction was obtained by unit gravity sedimentation. These blast cells were either cloned, as given below, or recultured and restimulated with the sensitising alloantigens one or more times and then used 2 days after

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Table 1 Proliferative response of non-cloned, PLT cells against agarose 'cloned' alloactivated cells

	Responders			
Stimulators	AB$_x$ PLT	Clone A31	Clone A17	Clone A11
HTC:				
DW1	475	NT	575	NT
DW2	708	215	711	506
DW3	478	480	696	604
DW4	281	NT	822	NT
DW5	824	NT	576	NT
DW6	1,031	368	3,031	NT
DW7	2,436	15,684	12,949	980
DW10	588	NT	674	NT
A	148	215	491	355
B	1,424	5,794	2,527	458

Ficoll-Hypaque purified peripheral blood lymphocytes from individual A (10^7) were mixed with an equal number of (4,000 rad) irradiated cells of individual B (AB$_x$) in Falcon 3013 flasks in 15% heat-inactivated human serum in RPMI-1640 with 25 mM HEPES (15% HS-RPMI). Following 1g sedimentation[12] on day 4 the 'blast' cell containing fractions were pooled and $2.5–5\times10^5$ cells in 1 ml 15% HS-RPMI mixed with 1 ml of TCGF containing agarose (Sea Plaque Agarose) to yield a predetermined, optimal concentration of TCGF and a 0.36% agarose concentration. This mixture was placed on a pre-gelled (2.5 ml) layer of 0.5% agarose in TCGF containing media in a 60-mm Petri dish, allowed to gel, and incubated in humidified 5% CO_2 in air at 37 °C until colonies of cells containing 20–100 cells appear. TCGF was always used at predetermined, optimal concentrations with 15% HS-RPMI (TCGF media) and was either prepared as described by Morgan *et al.*[7] or purchased as pooled units (Associated Biomedic Systems). Colonies were transferred after 7–14 days to round-bottom microtitre wells (Linbro 76–011–05) containing 0.15 ml of TCGF media and irradiated (4,000 rad) A or B lymphocytes as feeder cells ($5–10\times10^4$ per well). On days 5–6 of culture 50 µl of TCGF media is added to each well and after an additional 2 to 5 days of culture the amount of ^{3}H-thymidine incorporation was determined in 20-µl samples of each well. The contents of wells with dividing cells were transferred to 16×100 mm culture tubes or 16×17 mm wells (Linbro 76–033–05) containing TCGF media and $5–10\times10^5$ irradiated A or B feeder cells. Thereafter the pre-clones ('clones') were maintained at less than 10^6 cells per ml, replenished with fresh TCGF media every 2–4 days, and resupplied with feeder cells approximately every 10 days. Clones to be tested for PLT response (after washing to remove TCGF) as well as 10 day AB$_x$ PLT cells[4] were placed at a concentration of 2.5×10^4 cells per well in microtitre V-bottom plates (Linbro 76–021–05) containing 5×10^4 irradiated stimulating cells in 15% HS-RPMI and after 40 h the cultures were labelled with ^{3}H-thymidine for 8 h (A response is easily detectable at 24 h and peaks at 48 h). Values given are mean counts per minute of triplicate samples. NT, not tested.

restimulation for cloning. Cloning was attempted by two different methods. First, cells obtained from a primary MLC were placed, in the presence of TCGF, in soft agarose and 7 to 14 days later 'colonies' were removed and subsequently grown in microtitre wells in the presence of irradiated (feeder) lymphoid cells. Second, lymphocytes or cells from blast fractions from a primary MLC were placed at limiting dilutions in the wells of Terasaki microtitre plates in TCGF-containing medium also in the presence of feeder cells. One cannot be sure that the progeny obtained by either of these two methods are true 'clones' until recloning is done; although we have not, as yet, attempted re-cloning, for simplicity of expression we shall refer to the populations generated as clones.

Clones, obtained from MLC blast cells plated in agarose, were tested for reactivity to homozygous typing cells for the presently recognised HLA-DW clusters (Table 1). Of the clones that gave a proliferative response, all did so to the DW7 homozygous typing cell, as did the non-cloned AB$_x$ PLT cells which were collected and tested following the normal sensitisation time of 10 days[4,5]. In addition, some clones showed a weaker, but highly significant, response to DW6. Assuming that weak stimulation by the DW6 cells represents antigen sharing or cross-reactivity between DW7 and DW6, one would expect to find some clones that detect this 'shared' determinant; other clones should be specific for DW7. The results shown demonstrate that this is the case. In addition, these data provide evidence suggesting that HLA-D region-encoded determinants are important in the restimulation of the cloned PLT cells.

One of the 'clones' in Table 1 did not show any proliferative response to any restimulating cell tested. Cells of this clone were cytotoxically active against the original sensitising cell but not to targets autologous with the responding cells (Table 2). Agarose-cloned cells grown in TCGF are primarily T cells, 92% of which form sheep red blood cell (SRBC) rosettes while 64% of the non-cloned, AB$_x$ PLT cells (Table 1) rosette with SRBC.

Table 3 shows results obtained with cells of a number of different 'clones' obtained from a different primary MLC in terms of their PLT response either to restimulating cells from the sensitising cell donor, B, or to restimulating cells from individuals C and D, unrelated to B and the donor of the responding cells. Results obtained with the original PLT cell, AB$_x$, indicate that cells of C restimulate to a significant degree, although much less than do those of B; cells of D do not restimulate. Once again, assuming antigen sharing or cross-reactivity between B and C, one would expect to find clones, such as those represented by 40-8E and 40-4E, which give a highly significant response to restimulating cells of either B or C with still no response to cells of D. Other 'clones', such as 40-10E and 40-7E, retain specificity only for B.

Table 2 Cytotoxicity of cells from agarose clone A11 derived from alloactivated MLC blast population

	% Cytotoxicity (±s.d.) on target	
E:T	A	B
20:1	−8.4±9.3	43.1±11.0
5:1	NT	6.0±7.4
1.25:1	NT	−3.6±8.3

'Clone' A11 cells (see footnote to Table 1) were mixed at the given ratio with 1×10^4 ^{51}Cr-labelled target lymphocytes of the original responder (A) or of the original stimulator (B). After 8 h the amount of ^{51}Cr released was determined and per cent cytotoxicity calculated based on spontaneous and maximum release from the target cells in medium.

Response patterns of cells of these clones allow definition of HLA-encoded antigens with a degree of specificity previously unobtainable with the average PLT or homozygous typing cell reagent. Low levels of cross-stimulation by third party test cells of non-cloned, normal PLT reagents, a finding that has been very difficult to interpret in the past, in at least some cases apparently reflects antigen sharing or cross-reactivity. This conclusion is based on finding some clones of each PLT that are restimulated by only cells of one individual (with respect to those tested) and other clones from the same PLT that are restimulated by cells of that individual and by cells of another

Table 3 Proliferative response of non-cloned PLT versus limiting dilution 'cloned' alloactivated cells

	Restimulating cells			
Responding cells	A$_x$	B$_x$	C$_x$	D$_x$
Original PLT	173	5,152	831	207
'clones'				
40-10E	543	6,000	536	375
40-7E	641	44,681	906	489
40-8E	576	3,842	2,313	710
40-4E	814	4,634	2,475	683
40-9D	881	999	993	742

'Clones' were obtained from limiting dilution of day 4 MLC blast cells and were grown in Terasaki microtitre wells in the presence of feeder layers and TCGF. The cells were derived from wells which received on average 40 cells per well at the time of dilution into Terasaki plates. 40-9D is an example of a clone giving no proliferative response.

individual. As both HLA-D and DR antigens can restimulate PLT cells[9,10] it is most likely that cloning will allow fine structure dissection and analysis of the complex of HLA-D region-encoded antigens. PLT responses can be obtained with as few as 5×10^3 responding cells (ref. 11 and unpublished data); thus, cloning makes reagents available that could be used to type more than 1×10^4 individuals per clone. Although our emphasis has been on the testing of PLT reactive clones, the fact that cytotoxic T lymphocyte clones can also be obtained in these conditions, now makes possible certain experiments in man for the study of T lymphocyte function using homogeneous cell populations.

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Epidermal growth factor receptors increase during the differentiation of embryonal carcinoma cells

A. R. Rees,* E. D. Adamson† & C. F. Graham†

*Laboratory of Molecular Biophysics and †Department of Zoology, University of Oxford, South Parks Road, Oxford, UK

Mouse teratocarcinoma stem cells (embryonal carcinoma, or EC cells) bind very small amounts of mouse epidermal growth factor (EGF) and the latter hormone seems to have no stimulatory effect on the growth of two cloned lines of EC cells. However, when EC cells are induced to differentiate into large flat endodern-like cells (END cells), EGF receptors increase in number reaching a plateau in 6 to 8 days. At 8 to 10 days after induction, END cells multiply very slowly, but when EGF is added (3×10^{-10} M) to the medium, cell division is stimulated and a further change in morphology occurs. This letter describes the binding characteristics and numbers of the EGF receptors on EC and END cells and shows that exogenous retinoic acid increases the numbers of EGF receptors on END cells. We were unable to find endogenous competing factors produced by EC cells. Such factors could account for the lack of detectable binding of EGF on these cells. As EC cells differentiate to END cells, so the ability of the cells to form tumours is reduced[1,2]. Since this change is accompanied by an increase in the number of EGF receptors there may be a relationship between these two events.

The experiments were performed on the cloned cell lines PC13 clone 5 (ref. 3) and OC15S1 (ref. 4) which were both derived from the transplantable 129/JSv strain teratocarcinoma OTT6050 (ref. 5). Both clones are pluripotential; OC15S1 differentiates in culture to form muscle and nerve which express biochemical markers characteristic of these cell types[6,7], while

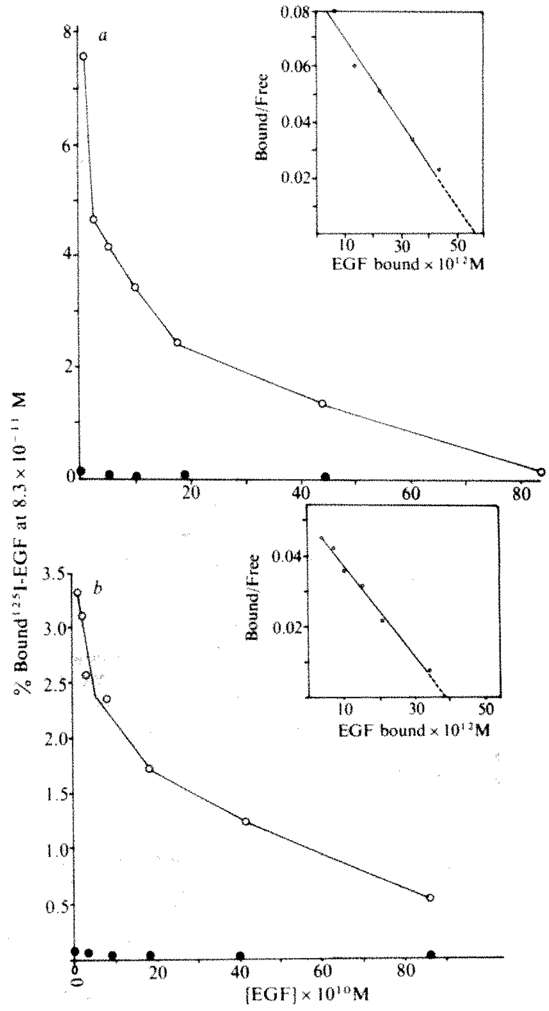

Fig. 1 Binding of ^{125}I-EGF to monolayers derived from *a*, PC13 clone 5 and *b*, OC15S1. EC cells were plated at 1×10^5 cells per 50-mm gelatinised dish in modified α-medium containing 10% fetal calf serum and 3×10^{-6} M retinoic acid[10]. Over 7 days with one change of medium, 1×10^6 END cells formed. Duplicate dishes at each point were used in binding assays. OC15 END cells were formed both as above and by the low-density plating method which does not require retinoic acid[9,10]. ^{125}I-EGF was prepared according to the following protocol: 4 μg EGF in 4 μl 0.3 M sodium phosphate buffer at *p*H 7.5 was added to 10 μl Na^{125}I (Radiochemical Centre, 100 mCi ml^{-1}). Chloramine-T 5 μl (2.5 mg ml^{-1} in water) was then added and the mixture agitated for 35–40 s after which 5 μl sodium metabisulphite (5 mg ml^{-1} in 0.3 M sodium phosphate buffer) and 100 μl bovine serum albumin (25 mg ml^{-1} in phosphate buffer) were added in quick succession. The ^{125}I-EGF was purified on a column (60 cm by 1.2 cm diameter) of Sephadex G-50 in phosphate-buffered saline containing bovine serum albumin at 1 mg ml^{-1}. Three peaks were obtained: the first contained damaged and over-iodinated material and the last free iodine. The middle peak (^{125}I-EGF) was split into 50-μl aliquots and frozen at −20 °C. The binding assay buffer contained Earle's balanced salt solution, HEPES (5 mM), and bovine serum albumin (1 mg ml^{-1}). Cell monolayers were washed twice with 2 ml buffer and then reacted with ^{125}I-EGF, finally 8.3×10^{-11} M, (970 Ci mmol^{-1} 250,000 c.p.m.) and with varying amounts of unlabelled EGF (from zero to 4×10^{-8} M) in a final volume of 2 ml buffer. The dishes were incubated at room temperature (~22 °C) for 90 min with intermittent shaking. The mixture was aspirated and the dishes were washed three times with ice-cold binding buffer. Cells were removed with 0.5 M NaOH and counted in a Hewlett–Packard γ-counter. The data points shown are the means of at least three separate determinations and values are normalised to 10^6 cells. Nonspecific binding was determined by measuring the bound counts in the presence of a large excess (520-fold over ^{125}I-EGF) of unlabelled EGF and was generally less than 5% of the specifically bound counts. ●, EC cells; ○, END cells. Inserts, Scatchard plots used to calculate binding constants.

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Table 1 Comparison of binding of ^{125}I-labelled EGF to various cell lines

Cell type	Additions	% EGF bound at 10^{-10} M per 10^6 cells	% EGF bound relative to PC13 END
PC13 EC	None	<0.1	<1.4
PC13 END	RA (1 d)	1.3	17.6
	RA (2 d)	3.2	43.2
	RA (5 d)	4.9	66.2
	RA (6 d)	7.2	97.3
	RA (8 d)	7.4	100.0
OC15 EC	None	<0.05	0.7
OC15 EC	RA (1 d)	0.15	2.0
OC15 END	None (7 d)	1.08	14.5
OC15 END	RA* (7 d)	3.31	44.7
PSA5E	None	1.3	17.6
PSA5E	RA (2 d)	1.25	16.9
PYS-2	None	<0.1	<1.4
PYS-2	RA (2, 4, 6 d)	<0.1	<1.4

The conditions for binding were as detailed in Fig. 1 legend. When retinoic acid was used it was added at 3×10^{-3} M in dimethyl sulphoxide (DMSO) to a final concentration of 3×10^{-6} M. As far as possible, cell densities were maintained at 10^6 cells per 50-mm dish. Separate experiments showed that the amount of ^{125}I-EGF bound increased linearly with cell density and that there was no decrease in binding as confluency was approached. The possibility that retinoic acid competed with ^{125}I-EGF for receptor sites was eliminated in separate control experiments. Addition of DMSO alone to EC cells affected neither the morphology of the cells nor the amount of ^{125}I-EGF bound.

* Retinoic acid was removed for 24 h before the assay.

PC13 forms these and other cell types when injected into adult hosts[8]. Both clones form large flat cells when grown in retinoic acid (3×10^{-6} M); and similar END cells are formed when OC15S1 cells are plated at low density and grown in a minimal medium[9,10]. Retinoic acid has been shown previously to induce EC cells to develop into endoderm-like cells which secrete plasminogen activator[11,12], type I-like collagen[10] and basement membrane protein[13].

EGF is a potent polypeptide mitogen extracted from mouse submaxillary glands (EGF, Collaborative Research). It interacts with specific cell-surface receptors on mouse and human fibroblasts, glial cells and corneal epithelial cells[14,15]. When mature END cells were incubated with ^{125}I-labelled EGF at 8.3×10^{-11} M in the presence of increasing concentrations of unlabelled EGF, specifically bound EGF was detected (Fig. 1). The data shown in Fig. 1 were used to estimate the binding association constants (K_a) and the numbers of EGF receptors per cell by Scatchard plots[16]. The value of 1.4×10^9 M^{-1} and 1.2×10^9 M^{-1} for PC13 END and OC15 END respectively are close to the K_a of 2.3×10^9 M^{-1} for human foreskin fibroblasts obtained by Carpenter and Cohen[1]. The latter cells have 10^5 EGF receptors per cell whereas END cells have slightly fewer: 7×10^4 for PC13 END and 3.1×10^4 for OC15 END. The number of EGF receptors in a mouse fibroblast line (STO[17]) was found to be similar to that in human fibroblasts; STO was therefore used as a positive control in these experiments.

The formation of PC13 END from EC cells takes place gradually on exposure to $3-6\times10^{-6}$ M retinoic acid, and the original small round EC cells form extremely large and flat cells (Fig. 2*a*, *b*, *c*). During the development of END cells there is some cell death[1] and this raises the possibility that during the formation of END cells, a minor subpopulation of EC cells with EGF receptors survives and overgrows during the END cell transition. However, this phenomenon is unlikely to explain our results with PC13 END cells because 24 h after the addition of retinoic acid, cell numbers had less than doubled while the amount of EGF bound had increased 12-fold. Table 1 shows that there is a gradual increase in the amount of EGF bound per cell during the transition; this is probably due to the gradual formation and maturation of END cells. PC13 EC and OC15S1 EC cells bound very little ^{125}I-EGF. It is clear from Table 1 that OC15 END cells which were formed in the presence of retinoic acid were able to bind higher levels of ^{125}I-EGF than similar END cells formed in the absence of exogenous retinoic acid. We also tested two teratocarcinoma-derived differentiated cell lines which were known to have endoderm-like properties. PSA5E cells[18] bound ^{125}I-EGF and binding was unchanged by the addition of retinoic acid; PYS-2 cells[19] did not bind significant amounts either in the presence or absence of retinoic acid. These cell lines are thought to possess endoderm properties similar to those of visceral yolk sac endoderm and parietal endoderm, respectively. This finding raised the possibility of differential distributions of EGF receptors in embryos and preliminary studies confirm this (manuscript in preparation).

Retinoic acid is required for the transition of PC13 EC into END cells, and promotes the formation of larger numbers of EGF receptors on OC15 END (Table 1). In contrast, a third effect occurs on removal of retinoic acid from PC13 (and OC15) END cells for various times before applying the ^{125}I-EGF binding assay. The number of EGF receptors on PC13 END cells was found to have increased to 28% above normal after 2 days culture in the absence of retinoic acid. Opposing effects of retinoic acid have also been documented in respect of its ability both to enhance tumour growth and to oppose promoters of tumour growth (see ref. 20 for review).

Figure 3 shows the effect of EGF on the growth of PC13 END cultured in 50-mm dishes seeded at 1×10^5 cells per dish. In one series EGF was absent and the cell number either decreased or remained the same; this depended on the age of the cells and the density of seeding but the continued presence of retinoic acid had no effect. In the other series, the addition of EGF at 2 ng ml^{-1} caused cell numbers to increase three- to fourfold in 6 days. The continued presence of retinoic acid appeared to be necessary to maintain this rate of cell division. OC15SI END cells responded similarly to EGF (not shown). After about 2

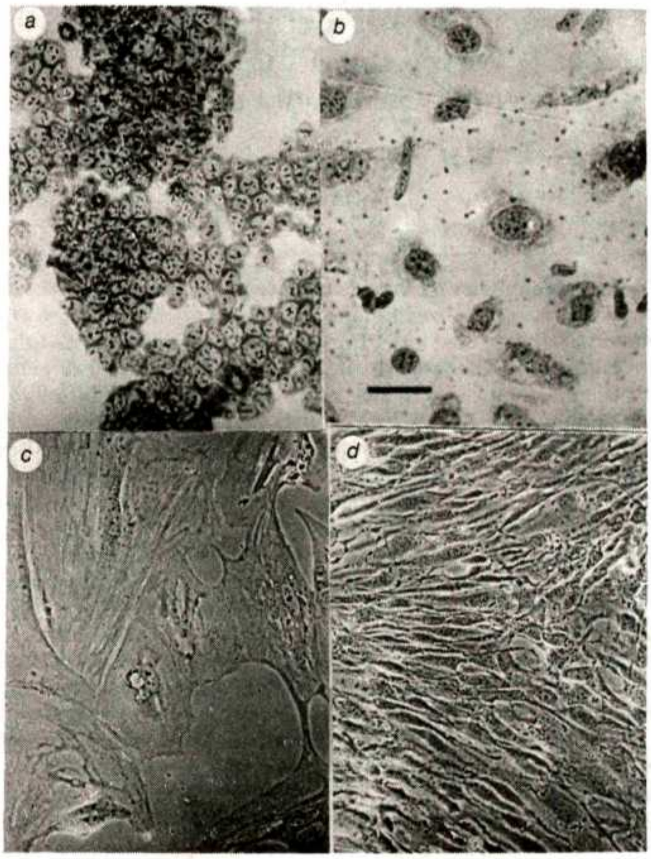

Fig. 2 Photomicrographs of; *a* EC cells; *b*, END cells stained with Giemsa; *c*, END cells; *d*, END cells after 4 days growth in EGF (3.7×10^{-10} M), phase contrast. All at the same magnification, scale bar, 50 μm.

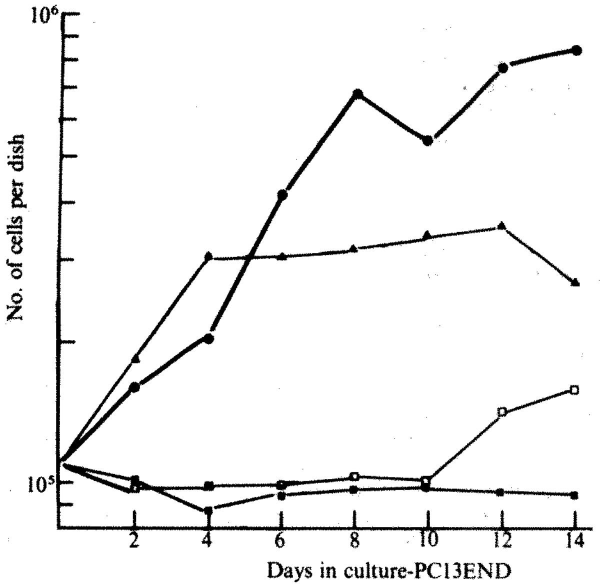

Fig. 3 Growth curves of PC13 END cells. END cells were formed as described in Fig. 1 legend and then reseeded at about 1×10^5 cells per 50-mm dish and cultured for two more days in medium containing 3×10^{-6} M retinoic acid. Medium was replaced with one containing 3.7×10^{-10} M EGF (▲); 3.7×10^{-10} M EGF and 3×10^{-6} M retinoic acid (●); with retinoic acid (■); or with neither (□). Cells in duplicate dishes were counted every other day and the medium was changed every 3 days. Similar results were obtained in each of three experiments with PC13 END and twice for OC15 END cells.

days in contact with EGF, PC13 END cells changed to a fibroblastic appearance (Fig. 2*d*) and later when more dense, they formed multilayers.

In a similar experiment the effect of EGF on the growth of OC15S1 and PC13 EC cells was tested (not shown). No effect was detected on the already fast multiplication rate of these cells (approximate doubling time 17 h). This finding was in accord with the very low binding of ^{125}I-EGF to EC cells shown in Table 1. It was possible that EGF receptors were present on EC cells but were masked by endogenous growth factors. Such factors have been described[21]: they were secreted by Moloney sarcoma virus (MSV)-transformed mouse cells; they had a mitogenic action on rat fibroblasts; and they competed with ^{125}I-EGF in the EGF receptor-binding assay. We have been unable to find any endogenous growth factors secreted into the medium or in the cytosol of PC13EC cells, but END cells shed a material which may contain EGF receptors since it inhibited the binding of ^{125}I-EGF to PC13 END cells by nearly 90%. During the incubation, END cells remained healthy and cell breakdown could not account for any release of proteases which might have affected the binding assay. It is also known that human lymphocytes shed insulin receptors when cultured in serum-free medium[22].

The main finding of these experiments is that both PC13 and OC15S1 EC cells, when allowed to transit into END cells, develop EGF receptors while also changing in morphology and in macromolecular secretory products[1]. We believe that the transition is a differentiative process and that an endoderm-like cell is the product[10-13]. END cells have a very low incidence of tumour formation: in contrast, EC cells are highly tumorigenic in adult hosts and therefore lack the growth controls which operate on normal cells. Hence the very low numbers of EGF receptors on EC cells and the very large numbers on END cells may indicate that EGF receptors are part of the growth control of these cells. Cells which are transformed by MSV[23] or by phorbol esters[24], have a decreased number of available EGF receptors (or reduced-affinity receptors[25]) and transformation can be blocked by retinoids[26]. It is possible that the blocking effect of retinoids is mediated through the renewed operation of EGF and the EGF-receptor system, since we show here that retinoic acid stimulates the production of EGF receptors. The experiments reported above demonstrate an increase in the numbers of EGF receptors with differentiation and the results are consistent with the hypothesis that teratocarcinoma tumour stem cells (and early embryonic ectoderm therefore) only become subject to growth control by EGF after their differentiation into cells of a more specialised and restricted type. It should be possible to test this hypothesis further.

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Sequential X chromosome inactivation coupled with cellular differentiation in early mouse embryos

Marilyn Monk & Mary I. Harper

MRC Mammalian Development Unit, Wolfson House,
4 Stephenson Way, London NW1, UK

Only one of the two X chromosomes is active in each somatic cell of adult female eutherian mammals, making the females (XX) equivalent to the males (XY) with respect to X chromosome dosage[1-4]. Biochemical analyses showing that both X chromosomes are active in female mouse embryos in mid-cleavage stage[4-8] indicate that X chromosome differentiation involves inactivation. This event occurs in most or all cells of the embryo at the blastocyst stage[4,7-9], when there are two cell types, the outer sphere of trophectoderm cells and the inner cell mass (ICM). Because there is genetic evidence that both X chromosomes are potentially active in ICM cells[10], it has been suggested that X chromosome inactivation has occurred in only the trophectoderm cells[9]. Further, one of us (M.M.)[4] has proposed that X chromosome differentiation is linked to cellular differentiation, occurring at different times in different cell populations as they 'depart' or terminally differentiate from a pluripotent fetal 'stem line' (Fig. 1). Analysis of a large number of inner cell masses isolated immunosurgically from female blastocysts has yielded data consistent with the presence of two active X chromosomes[11], but ICMs are so small that the biochemical assay used was at the limit of its accuracy. (Neverthe-

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less, a computer analysis of the data[8] indicated two ICM populations differing twofold with respect to X chromosome activity.) More tissue is available for analysis in post-implantation embryos, in which, on the above hypothesis, we would expect two active X chromosomes in the pluripotent epiblast region before gastrulation, but only one in the corresponding extra-embryonic ectoderm (a trophectoderm-derived tissue[12]) and primary endoderm (ICM-derived[12], see Fig. 1). We report here that this is the case; we also show that inactivation is complete in the epiblast (fetal precursor) cells between 6.0 and 6.5 d of gestation at the onset of gastrulation.

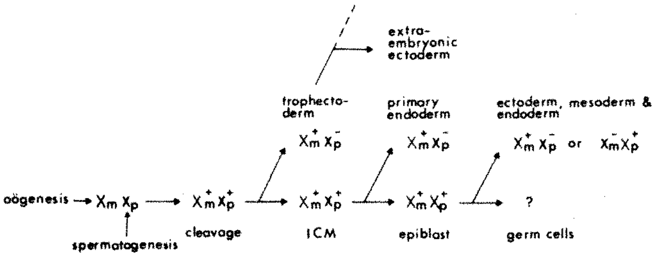

Fig. 1 Hypothesis linking X chromosome differentiation to cellular differentiation[4]. X chromosome differentiation is shown as occurring in different cell lineages as they differentiate (m, maternal; p, paternal; +, active; −, inactive). Tissues differentiating early, that is, trophectoderm and primary endoderm, show preferential inactivation of the paternally inherited X chromosome[16–18], suggesting some memory mechanism[2] detecting the parental origin of the two X chromosomes. This memory mechanism is presumably lost by the time of gastrulation when inactivation is random.

We used a biochemical assay to measure simultaneously the activities of two enzymes in dissected tissues of individual post-implantation embryos. These enzymes are the X-coded hypoxanthine phosphoribosyl transferase (HPRT, EC 2.4.2.8) and the autosomally coded[13] adenine phosphoribosyl transferase (APRT, EC 2.4.2.7). The assay has been previously described[6] (see Fig. 2 legend) and its validity recently unequivocally confirmed by thin layer chromatography (M.I.H. and M.M., unpublished). The results are expressed as a ratio of HPRT to APRT activities for a particular dissected tissue from an individual conceptus, thus eliminating sampling errors which would be introduced by variation in the amount of pure dissected tissues recovered. We expect the ratio of X-coded HPRT to autosomally coded APRT activity to be twice as high in an XX tissue as in an XY tissue if both X chromosomes are active in that particular region of the female conceptus.

Swiss female mice (MF1, Olac UK) were mated between 1100 and 1700 h to MF1 males in a reversed day/night room. The embryos were isolated from the uteri of these females 6 days later (6-d conceptuses), the trophectoderm and ectoplacental cone discarded and the egg cylinders dissected into three tissue populations: epiblast, extra-embryonic ectoderm and primary endoderm (see Fig. 2*a*). Extracts of these tissues were assayed for HPRT and APRT activity, and the ratios of activities of these enzymes per tissue per embryo are given in Fig. 2. It can be seen that whereas the values for the extra-embryonic ectoderm and primary endoderm regions (Fig. 2*c*, *d*) show unimodal distributions with a less than twofold spread, the values for the epiblast region (Fig. 2*b*) are bimodal. A computer analysis indicated two populations of epiblasts in the proportion 0.6 : 0.4 (lower : higher) and gave estimates compatible with a twofold difference between the means. By analogy with previous work[4,6,11], we assume that the bimodality is due to a bimodal distribution of X-coded HPRT activities. The results shown in Fig. 2 suggest that X chromosome inactivation has already occurred in extra-embryonic ectoderm and primary endoderm, whereas there seem to be two populations with respect to X chromosome activity in epiblast regions.

Several of these epiblasts were sexed by karyotyping their respective extra-embryonic regions. The results are shown in Fig. 3. The mean of HPRT/APRT ratios for the female epiblasts is significantly higher than that for male epiblasts (Student's *t*-test gives $t_{24} = 3.14$, $P < 0.01$). The fact that more epiblasts in Fig. 2 are located in the lower peak (see above) is consistent with the overlap between male and female values indicated in Fig. 3, and suggests that X chromosome inactivation has already occurred in about one-fifth of the XX epiblasts. One possible explanation for the bimodality in the epiblasts is that X chromosome inactivation has occurred at the same time as in primary endoderm, but that HPRT activity observed in 6-d epiblasts is due to enzyme which was present in the cells before this event. We consider this explanation unlikely because the 6-d epiblast is the most rapidly proliferating region of the embryo[14]. We do not know the spread in timing of the inactivation event in individual epiblast cells, but we can conclude that it occurs at around 6 days' gestation.

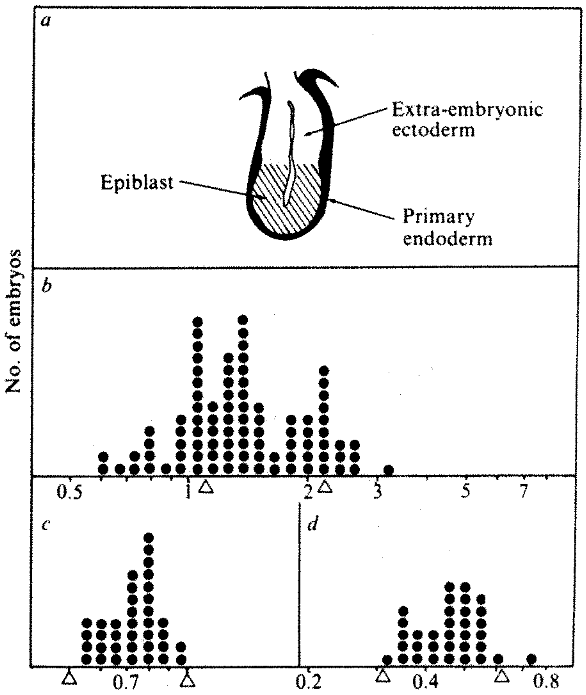

Fig. 2 Ratios of HPRT to APRT activities in dissected tissues of 6-d egg cylinders. *a*, Diagrammatic representation of 6-d egg cylinder; *b*, HPRT : APRT in individual epiblasts; *c*, HPRT : APRT in individual extra-embryonic ectoderm regions; *d*, HPRT : APRT in individual primary endoderm regions. The egg cylinders were isolated from decidua, washed in PB1 medium[24] containing 0.4% polyvinylpyrrolidone instead of albumin, and transferred to PB1 containing 0.5% trypsin and 2.5% pancreatin for 10 min at 4 °C. Enzyme treatment was terminated by the addition of fetal calf serum (to 2%). The egg cylinders were washed in PB1, the three components of each separated using finely drawn Pasteur pipettes and glass needles and transferred separately in 5 μl of medium to 10-μl microcaps. The microcaps were sealed and freeze-thawed three times in liquid nitrogen to lyse the cells. After centrifugation, the supernatants were transferred to 50 μl of reaction mix containing sodium phosphate buffer (35 mM, *p*H 7.4), magnesium chloride (5 mM), phosphoribosyl pyrophosphate (2 mM), ^{3}H-guanine sulphate (10 μM, specific activity 610 Ci mol^{-1}) and ^{14}C-adenine (10 μM, specific activity 286 Ci mol^{-1}). Reactions were carried out at 37 °C for 2 h. Further details are given in ref. 6. Arrowheads indicate a twofold span on the horizontal axis (logarithmic) for each distribution.

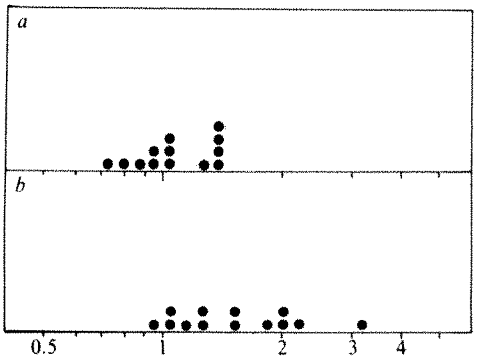

Fig. 3 Ratio of HPRT to APRT activities on 6-d male (*a*) and female (*b*) epiblasts. Embryo isolation and dissection, and enzyme assays were carried out as described in Fig. 2 legend. Individual extra-embryonic ectoderms were karyotyped as follows: tissues were incubated individually in droplets of Dulbecco's modified Eagle's medium (DMEM) containing 5% fetal calf serum and incubated overnight at 37 °C in a 5% CO_2-in-air atmosphere. They were transferred to the same medium containing 0.1 μg ml^{-1} colcemid and incubated for 4 h, followed by transfer to 1% sodium citrate for 45 min at room temperature, then to fresh fixative (3 : 1 ethanol/glacial acetic acid) at 4 °C for a minimum of 30 min. Spreads were made by transferring the tissue in a microdrop of fixative to a clean slide, promptly adding two droplets of 60% acetic acid and spreading on a hot plate at 60 °C. The preparations were stained in Giemsa diluted 1 : 10 in a phosphate buffer, *p*H 6.9, for 10 min. Sex determination was based on the presence of two versus three small chromosomes.

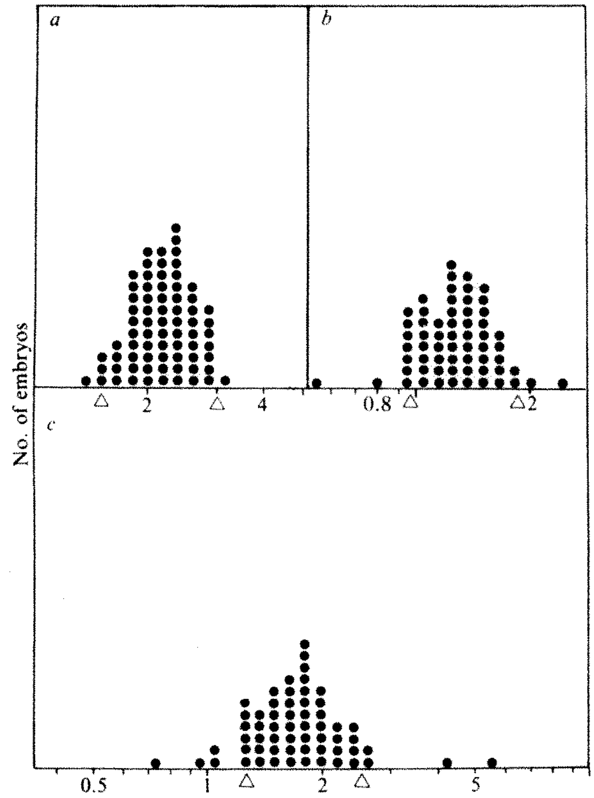

Fig. 4 Ratios of HPRT to APRT activities in dissected tissues of 6½-d egg cylinders. *a*, HPRT : APRT in individual epiblasts; *b*, HPRT : APRT in individual extra-embryonic ectoderm regions; *c*, HPRT : APRT in epiblast/HPRT : APRT in extra-embryonic ectoderm, per individual egg cylinder. Embryo isolation and dissection, and enzyme assays were carried out as described in Fig. 2 legend.

Twelve hours later in development there is no difference between the distributions of HPRT/APRT ratios in epiblasts and the other tissues (Fig. 4). Here, dissected epiblast and extra-embryonic ectoderm regions of 6½-d conceptuses (6½ d following mating in a normal day/night mouse room) show similar unimodal distributions which are incompatible with two populations with twofold differences in X-coded HPRT activity. The ratios of the HPRT/APRT values in epiblast to those of the corresponding extra-embryonic ectoderm are also convincingly unimodal (Fig. 4*c*). We conclude that X chromosome inactivation has occurred in epiblast regions by 6½ days' gestation. Kozak and Quinn[15] analysed individual post-implantation embryos for the X-coded phosphoglycerate kinase (PGK, EC 5.3.1.1). They failed to detect a bimodal distribution of (presumably) whole egg cylinders (epiblast + extra-embryonic ectoderm + endoderm), but the embryos they analysed seem to have been more closely equivalent to our 6½-d embryos.

Our findings provide strong evidence for the hypothesis outlined in Fig. 1 that X chromosome differentiation in early development is linked to cellular differentiation. The activity status of the X chromosomes in the germ cell line is not clear. It is of interest that tissues arising from trophectoderm and primary endoderm isolated from later post-implantation stages show preferential inactivity of the paternally inherited X chromosome[16–18]. Cells differentiating early in development seem to have some memory mechanism which recognises the origins of the two X chromosomes.

The coupling of X chromosome differentiation with cellular differentiation may mean that the mechanisms involved in 'switching-off' the whole X chromosome are similar to those which select activity or inactivity in different autosomal regions characteristic of specialised cell function. Ohno[19] has suggested that similar basic mechanisms might be expected for X-inactivation and cellular differentiation. However, attempts to examine X-inactivation by reversing the process have yielded little information; apart from localised derepression[20], strong selective pressures have failed to reverse the inactive state of the silent X chromosome[21,22]. Martin *et al.*[23] have shown that X chromosome inactivation occurs with *in vitro* differentiation of an XX teratocarcinoma cell line. One of the implications of their findings, and ours, is that two active X chromosomes may be incompatible with the differentiated state.

We thank Hugh Paterson for help with the embryo dissections shown in Fig. 4, Anne McLaren for verifying the karyotypes of the samples in Fig. 3 and for her critical reading of the manuscript, and R. Cuthbertson for computer analysis of the data.

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Zoological origin of gonadotropin subunits and association kinetics

Jacques Marchelidon*, Roland Salesse†, Jean Garnier†, Elisabeth Burzawa-Gerard* & Yves-Alain Fontaine*

* Laboratoire de Physiologie générale et comparée du Muséum, Laboratoire d'Endocrinologie comparée associé au CNRS, 7 rue Cuvier, 75005 Paris, France.
† Laboratoire de Biochimie physique, INRA, Service de Biologie physico-chimique, Bât. 433, Université de Paris-Sud, 91405 Orsay, France.

Mammalian luteinising hormone (LH) is an association of two dissimilar subunits, α and β. *In vitro* studies, mainly using difference spectrophotometry, had shown that this phenomenon was slow, especially at low temperatures. If the situation was the same in poikilotherms, it would probably make gonadotropin (GTH) synthesis difficult for these animals in a cold environment. We have found that the formation of a teleost gonadotropin is in fact strikingly more rapid and less thermodependent than formation of mammalian LH. Also, studies with a fish–mammal hybrid molecule have allowed us to estimate the respective influence of the α and β subunits in determining these differences.

Association of mammalian LH subunits is a very slow process. Various methods of study have given convergent results indicating that maximal association is not attained before several hours at 37 °C, or several days at 20 °C[1-3]. Indeed the phenomenon is highly thermodependent[3,4]; at 0 °C no significant association was observed after 6 hours[3].

It became possible to investigate this problem in a poikilotherm when α and β subunits of GTH from a teleost fish, the carp (*Cyprinus carpio L.*) were isolated, and shown to reassociate[5]. Carp GTH(c-GTH) and its subunits were prepared according to Burzawa-Gerard[5] and dissolved in 0.02 M phosphate buffer *p*H 7.4 containing 0.1 M KCl. Carp GTH α and β were mixed in equimolar amounts assuming respective molecular weights of 14,000 and 17,000[6]. Subunit association was followed by difference spectrophotometry in conditions as used previously for the study of ovine LH (o-LH) formation[2,7] at three concentrations of each subunit (1.9, 3.6 and 6.8 μM, recalculated from absorbance at 280 nm). At 20 °C and for a concentration of 6.8 μM, the percentage association was 75% after only 45 min of incubation and was still clearly increasing at this time[8]. This indicated that virtually all the subunits were able to associate, and this was confirmed by bioassay; indeed a 60–100% recovery of biological activity (measured by the frog spermiation test) was obtained, depending on the conditions of incubation[9].

Figure 1 shows the results obtained at 4 °C. When the inverse of the concentration in free subunits was plotted as a function of time, parallel straight lines were obtained with the three different initial concentrations of subunits, indicating an apparent second order reaction with a rate constant (k) equal to $78 \pm 9\ \mathrm{M}^{-1}\ \mathrm{s}^{-1}$. Confirming this, a first order plot was nonlinear with estimated rate constants depending on subunit concentration. At 20 °C, the results (not shown) were qualitatively similar to those at 4 °C but k was $149 \pm 24\ \mathrm{M}^{-1}\ \mathrm{s}^{-1}$. Between 4 and 20 °C the Q_{10} of the reaction was 1.5 (limits for $P = 0.05$: 1.44–1.57). These values are strikingly different from those measured for o-LH (k 20 °C $= 0.86\ \mathrm{M}^{-1}\ \mathrm{s}^{-1}$; $Q_{10} = 4.8$ between 10 and 37 °C)[4]. Indeed, even at the low temperatures at which fish tend to live, the formation of GTH from its subunits may occur at a reasonable rate. Note that the changes in the circular dichroism spectrum seen during hormone formation from subunits are very similar for c-GTH and for o-LH[7]. As for mammalian hormones, thermal perturbation spectroscopy of c-GTH showed that three tyrosine residues (3.2 ± 0.2) were shielded from the solvent when the subunits were associated. These data show that similar structural changes occur during the formation of c-GTH or mammalian LH from their subunits, although these associations proceed at markedly different rates.

Table 1 Thermodynamic parameters for the formation of c-GTH, hybrid b-LH α–c-GTH β, and o-LH from subunit components at 20 °C

	Enthalpy (ΔH^*, kcal M^{-1})	Entropy (ΔS^*, cal $\mathrm{K}^{-1}\ \mathrm{M}^{-1}$)	Free enthalpy (ΔG^*, kcal M^{-1})
c-GTH	+5.38	−30.1	+14.2
Hybrid	+6.62	−31.3	+15.80
o-LH†	+23.5	+21.5	+17.2

The thermodynamic parameters were calculated by plotting ln k against $1/T$ (T, temperature in K). Measuring the slope of the curve ($-(E/R)$, where E is the energy of activation) we obtained $\Delta H^* = E - RT$, then $\Delta G^* = RT(\ln KT/h - \ln k)$ and $\Delta S^* = \Delta H^* - \Delta G^*/T$ (K, Boltzmann constant; h, Planck constant).

† Data calculated from ref. 4.

An attempt was made to determine the respective roles of α and β subunits in the important differences observed between the formation kinetics of c-GTH and o-LH. To this end, we took advantage of the fact that bovine (b)-LH α and c-GTH β are able to associate and to form a hybrid molecule possessing a very high biological activity on frog spermiation[10]. Bovine LH α and c-GTH β were mixed in equimolar amounts and the association process was followed by difference spectrophotometry at two concentrations (3.3 and 6.3 μM) and two temperatures (4 and 20 °C (Fig. 2)).

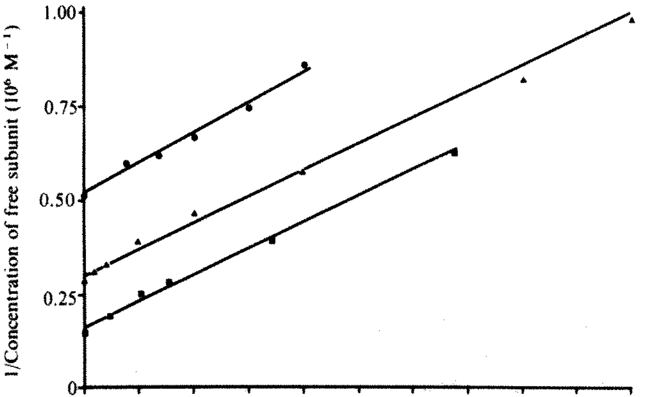

Fig. 1 Second order rate plot of the association of c-GTH subunits at 4 °C. Equal volumes (680 μl) of α or β subunits solutions were placed into the compartments of a double-sector cell. Two such cells were prepared. After equilibration of the temperature (zero time) mixing was accomplished in one of them (sample cell), the other, unmixed, cell being used as reference. The difference in absorbance at 287 nm was scanned at various times on a Cary 118 spectrophotometer. Separately, the maximum absorbance difference (ΔA_{max}) corresponding to a 100% unfolding of c-GTH was measured between two solutions of native hormone at the same concentration (8.6 μM), one of which was acidified at *p*H 2.0 with 5 M HCl (complete unfolding of c-GTH); the ratio of ΔA_{max} to the A_{280} of the native hormone solution was 0.11. The percentage of association at each time was then calculated as the ratio: ΔA_{287} between mixed and unmixed cells/$0.11 \times A_{280}$ of the native hormone solution at the concentration under study. The concentration of free, native subunits was determined from this percentage by assuming that all the subunits present at zero time were able to associate (see text). The inverse of the free subunit concentration was plotted as a function of time. Experiments were carried out at three subunit concentrations: 1.9 μM (●), 3.6 μM (▲) and 6.9 μM (■). The correlation coefficient was >0.99 in each case.

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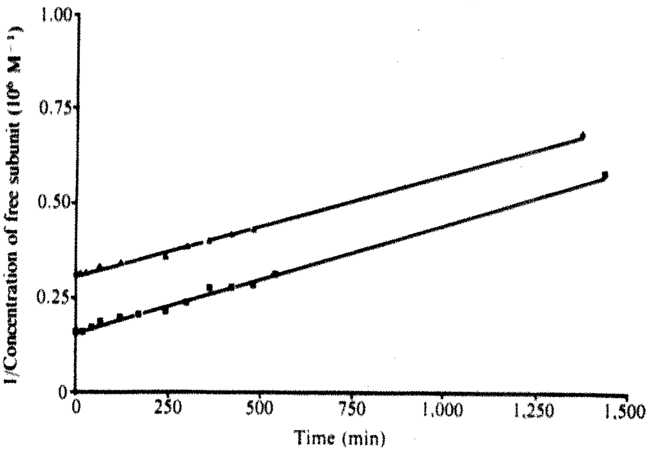

Fig. 2 Second order rate plot of the formation of hybrid b-LH α–c-GTH β from subunits at 4 °C. Association was followed as described in Fig. 1. The maximum absorbance difference corresponding to a 100% unfolding was measured between two solutions of purified hybrid at 6.2 μM, one of which being acidified at *p*H 2.0 with HCl 5 N; the ratio of ΔA_{max} to A_{280} of the hybrid solution was 0.094. Purified hybrid was obtained as follows; a mixture of b-LH α and c-GTH β (124 μM each) was incubated for 16 h at 20 °C, gel-filtered on Sephadex G-100 in NH_4HCO_3 0.1 M; the fractions with an exclusion coefficient from 0.1 to 0.32, corresponding to a protein peak with a molecular weight close to 30,000, were pooled and lyophilised. Calculations were carried out as indicated in Fig. 1 legend. The inverse of the free subunit concentration was plotted as a function of time. Association kinetics were studied at two subunit concentrations: 3.3 μM (▲) and 6.3 μM (■). The correlation coefficient was >0.99 in each case.

At 20 °C and for a concentration of 6.3 μM, the percentage of association was about 80% after 900 min and it was still clearly increasing at this time. Bioassays confirmed that virtually all the b-LH α and c-GTH β molecules were able to associate: a mixture of these two subunits (62 μM each) was incubated for 6 h at 25 °C; then the dose inducing 50% positive responses (D50) in the frog spermiation test was 0.15 μg (0.09–0.25) not different from the D50 for purified hybrid prepared as described in the Fig. 2 legend.

As for c-GTH subunits, the kinetics of association of b-LH α and c-GTH β indicated an apparent second order rate reaction at both 4 °C (Fig. 2) and 20 °C (data not shown). The *k* values were 4.8 $M^{-1}\,s^{-1}$ (5.0–4.6) at 4 °C and 9.8 $M^{-1}\,s^{-1}$ (9.7–9.9) at 20 °C; the Q_{10} within this interval was 1.5.

Thermodynamic parameters for the association reaction were calculated and are presented in Table 1. Because of the complexity of the mechanisms of association, and as shown with o-LH[4], no straightforward interpretation of these parameters can be proposed. However, it is clear that the rate of the hybrid formation is intermediate between those for the two mother hormones whereas the activation enthalpy is very close to that for the fish hormone.

Thus differences in the structure of the α subunit do not seem to modify the influence of temperature on the association rate: the thermodependence of the reaction is determined solely by the β subunit. In contrast, not only the β subunit (as suggested from work on mammalian hormones[11]) but also the α subunit, contribute to determine the association rate at a given temperature.

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GABA-mediated biphasic inhibitory responses in hippocampus

B. E. Alger & R. A. Nicoll

Departments of Pharmacology and Physiology, University of California, San Francisco, California 94143

γ-Aminobutyric acid (GABA) can have a biphasic effect on membrane potential when applied to certain central nervous system neurones[1–3]. Typically with iontophoresis, this effect is a negative–positive sequence of deflections and both phases are associated with an increase in conductance. However, iontophoretic application of neurotransmitter substances can produce effects even in cases where ordinary synaptic transmitter release is impossible; for example, on dorsal root ganglion cells. Thus, it is important in assessing the physiological relevance of the depolarising GABA response to determine if similar actions are produced synaptically. In addition, although the depolarising aspect of the GABA response has been attributed to activation of dendritic receptors, this identification has remained uncertain. Here, we have used the hippocampal slice preparation to demonstrate that a biphasic inhibitory response results from orthodromic, but not antidromic electrical stimulation in the presence of anaesthetic concentrations of pentobarbital. (For the sake of brevity, we use the term 'biphasic inhibitory postsynaptic potential (i.p.s.p.)' to describe the response complex, although it seems likely that a combination of two distinct events, rather than two parts of a single event, occurs.) GABA antagonists and tetrodotoxin (TTX) were used to show that the depolarising phase is due to synaptically released GABA acting on dendritic sites in the CA1 region. Both negative and positive phases are associated with a large conductance increase, and both block cell firing, indicating their inhibitory nature. The ionic mechanism of the depolarising phase is not fully understood but seems to involve a chloride conductance increase. Although the functional significance of the depolarising sign of the i.p.s.p. is not clear, the dendritic location would provide very effective control over dendritic excitability.

The slice preparation we use has been described elsewhere[4]. The major modification adopted here is that slices are held fully submerged between two pieces of nylon mesh. This arrangement permits prolonged stable recordings and the evaluation of drug effects from a single cell in both control and drug conditions. To oxygenate the slices adequately, the temperature of the bathing medium was held at 28–30 °C (ref. 5). The medium consists of 116.4 mM NaCl, 5.37 mM KCl, 2.5 mM $CaCl_2$, 1.3 mM $MgSO_4$, 0.92 mM Na_2PO_4, 26.2 mM $NaHCO_3$ and 11 mM glucose. Forty-three cells from male Sprague-Dawley rats were selected for analysis. All impalements were maintained for at least 30 min with cell resting membrane potentials over 50 mV (mean 58.1, s.d. 5.2 mV), action potential amplitudes of 78.5 ± 11.0 mV and total neurone input impedances of 45.8 ± 17.0 MΩ. Conventional intracellular recording techniques were used.

Electrical stimulation of CA1 stratum (s.) radiatum produces an excitatory postsynaptic potential (e.p.s.p.) followed by a long hyperpolarising i.p.s.p. in CA1 pyramidal cells. If 10^{-4} M pentobarbital (a concentration within the range occurring during

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anaesthesia) is added to the medium, the hyperpolarisation is prolonged and usually followed by a depolarisation (5.5 ± 3.5 mV) lasting seconds (7.9 ± 3.3 s, $n = 18$; Fig. 1*a*). The depolarisation is more reliably elicited with strong rather than weak (near threshold) stimuli. Passage of a series of 100-ms current pulses across the membrane shows that the entire sequence is accompanied by a drop in membrane resistance (Fig. 1*b*).

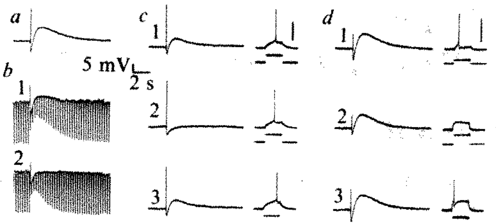

Fig. 1 Orthodromic production and dendritic localisation of depolarising i.p.s.ps in CA1 pyramidal cells. *a*, Orthodromic stimulation (s. radiatum) elicits hyperpolarisation followed by prolonged depolarisation. Resting potential (RP) −57 mV. *b*, Orthodromic and antidromic stimulation compared in a single cell. Downward deflections produced by hyperpolarising 100-ms current pulses (2 Hz) through a bridge circuit. Bridge balance was good. Reduced membrane resistance accompanies both responses; however, no depolarisation is seen with antidromic stimulation. RP, −59 mV. *c*, TTX (10^{-5} M) injected in the dendritic region abolishes depolarisation with no effect on hyperpolarisation. Action potentials produced by current pulses were unaffected immediately after TTX injection (c_2) although depolarisation was eliminated. The spike was subsequently blocked but recovered (c_3). RP, −63 mV. *d*, TTX injection in the somatic region greatly attenuates hyperpolarisation and blocks direct action potentials with little effect on depolarisation (d_2). Both hyperpolarisation and action potential recovered substantially (d_3). RP, −56 mV. All records were obtained in the presence of 10^{-4} M pentobarbital. Calibration for pen traces is 5 mV and 2 s. Calibration for oscilloscope traces is 40 mV and 1.0 nA. Current pulse duration is 100 ms in *c*, 30 ms in *d*.

GABA involvement in the depolarising phase is suggested by the observation that pentobarbital, which selectively enhances GABA action, is necessary to elicit it electrically. Further, perfusates containing GABA antagonists picrotoxin (10^{-5} M) or bicuculline (10^{-5} M) block the response ($n = 4$; data not shown) and, as seen below, iontophoretically applied GABA can mimic both hyper- and depolarising phases. These results strongly implicate GABA as the neurotransmitter for the biphasic action.

With iontophoretic pipettes independently positioned 200 µm within the slice at mid-dendritic and somatic regions, recording from a single cell shows that, even in the absence of pentobarbital, dendritic application of GABA produces a depolarisation whereas somatic application yields a hyperpolarising–depolarising sequence (Fig. 2*c*), as previously shown by others[2]. The interpretation of this pattern included the postulation of dendritic GABA receptors. Indeed, we have found that the depolarising action of GABA persists after synaptic transmission has been eliminated by changing to a low Ca^{2+} saline containing 8 mM Mg^{2+} ($n = 4$; data not shown), demonstrating that it is produced by receptors on the pyramidal cells themselves and is not an indirect effect. We hypothesised, therefore, that the electrically evoked, GABA-mediated depolarisation described above might represent a dendritic potential. To test this, we injected TTX (10^{-5} M) through a broken pipette, into the CA1 apical dendritic region. In four injections with three cells in which a large hyperpolarising–depolarising response was present, TTX selectively blocked the depolarising phase and its underlying conductance increase, but had little effect on the hyperpolarising i.p.s.p. or the action potential produced by somatic current injection (Fig. 1*c*). Neither resting potential nor input impedance changed and the TTX effect was reversible. The finding that the somatic spike was not altered at a time when the depolarising phase was blocked indicates that the depolarisation requires neuronal activity in the dendritic region. In the converse experiment, TTX injection through a pipette carefully positioned near the soma resulted in a complete block of somatic action potentials and substantial reduction in stimulation-evoked hyperpolarisation, without major effect on the depolarisation ($n = 4$; Fig. 1*d*). (Coarse TTX injections in the somatic, but not the dendritic, regions often resulted in the simultaneous reduction of both phases which may mean the cell whose synapses produce the depolarisation exists in or near s. pyramidale.) Taken together, the results with TTX injections imply that orthodromic stimulation in s. radiatum activates dendritic synapses which mediate i.p.s.ps having a depolarising component.

Both phases of the orthodromically activated response seem to exert an inhibitory influence on the pyramidal cell as measured by their ability to block action potentials caused either by intrasomatic depolarising current pulses or by e.p.s.ps (Fig. 2*a*,*b*). Similar blocking action is seen following iontophoresis of GABA on the dendrites (Fig. 2c_2,c_3).

It was often possible to see in a single cell a biphasic response to orthodromic (s. radiatum) stimulation and hyperpolarisation of shorter duration to antidromic (alveus) stimulation, the latter closely resembling the response to orthodromic stimulation following TTX application to the dendrites (compare Fig. 1b_2 and c_2). With very high intensity stimulation, small depolarisations could occasionally be elicited with antidromic stimuli, but current spread to known orthodromic fibres in nearby s. oriens[6] could not be ruled out in these cases. These findings indicate that orthodromic stimulation, in addition to activating the well documented recurrent inhibitory pathway[7], also activates a distinct set of inhibitory neurones which synapse on the dendrites of pyramidal cells and can have a depolarising action. We assume that pentobarbital merely emphasises a response which is normally present. That this drug is required to observe the depolarising i.p.s.p. may be due to the marked prolongation of GABA action which would result in a partial temporal

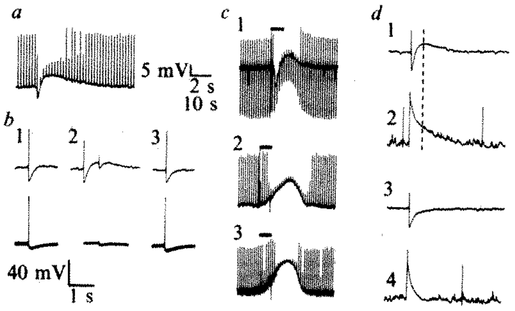

Fig. 2 Inhibitory effectiveness and chloride dependence of depolarising i.p.s.p. *a*, A train (2 Hz) of depolarising current pulses initiated full-sized somatic action potentials (amplitudes not accurately reproduced by pen recorder) which were blocked during most of the stimulation-evoked response. *b*, Inhibition of spikes elicited by just supra-threshold e.p.s.p. during a depolarising i.p.s.p. (b_2). Unconditioned control responses shown before and after inhibition (b_1 and b_3). Same cell as in *a*. RP, −59 mV. *c*, Effects of iontophoretic GABA (1 M) (horizontal bar) in normal medium without pentobarbital. c_1, Application through pipette in s. pyramidale. 100-ms current pulses given at 1 Hz (see Fig. 1b_1). RP, −55 mV. c_2, c_3, GABA iontophoresed in s. radiatum region of another cell. During depolarisation, both directly and synaptically activated action potentials are blocked (c_2 and c_3, respectively). RP, −61 mV. Iontophoretic pipettes were lowered independently 200 µm into the slice before impaling a cell. Ejection currents were 500 nA for 5 s. *d*, Two cells from the same slice in pentobarbital. d_1, Recorded with a 2 M potassium methylsulphate-filled pipette; d_2, with a 3 M KCl pipette. Note large amplitude and prolonged time course of depolarising response in d_2 after Cl$^-$ leakage into the cell. Small reversed i.p.s.ps visible on the baseline of the KCl-filled cell are capable of triggering action potentials. Antidromic responses from the same cells are shown for comparison (d_3, d_4). RP, −59 mV (d_1, d_3); −56 mV (d_2, d_4). Calibration for pen traces in *a*, *b* and *d* is 5 mV, 2 s; in *c* it is 5 mV, 10 s.

separation of the somatic and dendritic components. Assessment of the physiological role of the depolarisation will require more information on this point. The depolarising dendritic action of GABA in the absence of pentobarbital strengthens the notion that the dendritic i.p.s.p. normally has a depolarising component. A depolarising i.p.s.p., which is markedly enhanced by pentobarbital, has been reported to occur in olfactory cortical neurones[8].

The ionic mechanisms of the biphasic i.p.s.p. are not entirely understood. However, elevation of intracellular chloride by leakage from a 3 M KCl electrode reverses the hyperpolarisation and increases the positive phase of the i.p.s.p. ($n = 4$; Fig. 2*d*). This finding, in addition to the large conductance increase, suggests that chloride is at least partially involved in both phases. To account for the depolarising sign of the dendritic i.p.s.p., it must be assumed either that the chloride equilibrium potential is positive relative to the dendritic membrane potential, or that the conductance increase also involves other ions such as sodium or calcium. These cations alone could not be responsible for the depolarisation as excitatory, perhaps regenerative, activity would be expected to occur with a large increase in their conductances. Chloride plus either sodium or calcium currents could easily produce the observed phenomena. (Experiments like that of Fig. 2*b* imply that the depolarisation cannot be due simply to a depolarising shift in the local i.p.s.p. equilibrium potential, as in that case the superimposed test i.p.s.p. (Fig. 2b_2) would not be hyperpolarising.) Interestingly, Dudel has recently reported[9] that the inhibitory postsynaptic currents in crayfish muscle have a fast chloride conductance phase followed by a slower conductance phase which may involve an appreciable contribution of cations.

We have confirmed the presence of GABA-mediated dendritic i.p.s.ps and presented evidence suggesting these form an inhibitory system distinct from the classical recurrent pathway. We have demonstrated further that there is a depolarising aspect to the synaptically activated potential in pentobarbital which seems peculiar to the dendritic region. Differences in experimental species, temperature and recording conditions may all contribute to the fact that previous investigations *in vivo* did not report the depolarising component.

Although it seems clear that selective orthodromic activation of a dendritic i.p.s.p. would provide optimal control over local dendritic events[10], the functional significance of the depolarising phase is unknown. It is apparent that the pronounced conductance increase accompanying this depolarisation can predominate in inhibiting action potential generation. It remains possible, however, that the depolarisation could act to reduce the inhibitory effectiveness of the conductance increase.

The inhibitory neurones involved in the dendritic i.p.s.p. pathway have not been identified, although few cell bodies are present in s. radiatum. Possible candidates are the non-pyramidal cells which have somata near s. pyramidale and vertically orientated axons branching in s. radiatum[11-14].

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1α,25-Dihydroxyvitamin D_3 and 24,25-dihydroxyvitamin D_3 *in vitro* synthesis by human decidua and placenta

Y. Weisman[*] & A. Harell[*], S. Edelstein[†], M. David[‡], Z. Spirer[§] & A. Golander[§]

[*]Vitamin Research Laboratory, Ichilov Hospital, Municipal-Governmental Medical Centre, Tel Aviv, Israel
[†]Biochemistry Department, Weizmann Institute of Science, Rehovot, Israel
[‡]Maternity Hospital, Municipal-Governmental Medical Centre, Tel Aviv, Israel
[§]Department of Pediatrics, Rokach Hospital, Municipal-Governmental Medical Centre, Tel Aviv, Israel

The biologically active metabolite of vitamin D, 1α,25-dihydroxyvitamin D ($1\alpha,25(\mathrm{OH})_2\mathrm{D}$), is synthesised by successive hydroxylation of vitamin D in the liver and kidney[1]. The latter organ is the only known site of 1α-hydroxylation of 25-hydroxyvitamin D (25-OH-D)[2] and is also a major site of production of 24,25-dihydroxyvitamin D ($24,25(\mathrm{OH})_2\mathrm{D}$), a metabolite involved in bone ossification[3]. However, 24-hydroxylation of 25-OH-D may also occur in rat intestinal homogenates and in cartilage cells[4,5]. Accumulating data show that, during pregnancy, $1\alpha,25(\mathrm{OH})_2\mathrm{D}$ increases in the maternal circulation[6] but is absent or present only in small amounts in the fetus[7,8]. In comparison, $24,25(\mathrm{OH})_2\mathrm{D}$ accumulates in fetal tissues, particularly in the skeleton[8]. Little is known, however, about the independent metabolism of vitamin D in the fetoplacental unit. It has been reported[9] that the nephrectomised pregnant rat can synthesise $1\alpha,25(\mathrm{OH})_2\mathrm{D}_3$ and $24,25(\mathrm{OH})_2\mathrm{D}_3$ and that the fetoplacental unit is the most likely site of production of such metabolites. Those data suggested that there may be independent vitamin D metabolism in the fetoplacental unit. We now report *in vitro* synthesis of $24,25(\mathrm{OH})_2\mathrm{D}_3$ by human placenta and of $1\alpha,25(\mathrm{OH})_2\mathrm{D}_3$ and $24,25(\mathrm{OH})_2\mathrm{D}_3$ by human decidua. The human decidua is a development of the endometrium that is unique to pregnancy, originating from both epithelial and stromal cells and intimately attached to fetal structures, that is, the placenta and chorion.

Human placental, decidual and amniotic tissues from uncomplicated, full-term and from 8–12-week pregnancies were obtained within minutes of delivery or legal abortion and prepared for culture as described previously[10,11]. Explants of the tissues were cultured with RPMI 1640 medium (GIBCO) for 48 h and then incubated with tritiated 25-OH-D_3 for 24 h as described in Fig. 1 legend. At 72 h, the tissues and medium were extracted with chloroform–methanol[12]. The radiolabelled metabolites of vitamin D_3 in the lipid extracts were separated on columns of Sephadex LH-20 (Pharmacia), calibrated with authentic tritiated metabolites and developed with chloroform–petroleum ether (65 : 35, v/v) (Fig. 1).

Cultures of both full-term and young placental tissue synthesised $24,25(\mathrm{OH})_2\mathrm{D}_3$. The mean (±s.d.) percentage of tritium recovered from the Sephadex LH-20 columns as $^3\mathrm{H}$-$24,25(\mathrm{OH})_2\mathrm{D}_3$ was 6.97 ± 0.18 (full-term placenta) and 6.82 ± 1.23 (young placenta). Cultures of both full-term and young decidual tissue synthesised $1\alpha,25(\mathrm{OH})_2\mathrm{D}_3$ and $24,25(\mathrm{OH})_2\mathrm{D}_3$. The mean (±s.d.) percentage of tritium recovered from the columns as $^3\mathrm{H}$-$1\alpha,25(\mathrm{OH})_2\mathrm{D}_3$ was 3.65 ± 0.31 (full-term decidua) and 1.03 ± 0.61 (young decidua), and the percentage of tritium recovered as $^3\mathrm{H}$-$24,25(\mathrm{OH})_2\mathrm{D}_3$ was 3.12 ± 0.46 and 3.83 ± 0.41, respectively. Amniotic tissue did not synthesise either $1\alpha,25(\mathrm{OH})_2\mathrm{D}_3$ or $24,25(\mathrm{OH})_2\mathrm{D}_3$.

The identity of the putative $24,25(\mathrm{OH})_2\mathrm{D}_3$ and $1\alpha,25(\mathrm{OH})_2\mathrm{D}_3$ was established when samples from the fractions

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eluting from the Sephadex LH-20 columns in the positions of $24,25(OH)_2D_3$ and of $1\alpha,25(OH)_2D_3$ were applied to a high pressure liquid chromatography system (Fig. 2). The identity of the putative $24,25(OH)_2D_3$ was further defined by subjecting the $24,25(OH)_2D_3$ region to the periodate cleavage reaction[13], which resulted in the loss of radioactive label for the metabolite. In one experiment, 0.3 μg $[1,2\text{-}^3H,4\text{-}^{14}C]$25-OH-$D_3$ ($^3H/^{14}C$ radioactivity ratio 4.9:1, isolated from plasma of vitamin D-deficient chicks injected with $[1,2\text{-}^3H,4\text{-}^{14}C]$vitamin D_3) was incubated with pieces of decidua and the putative $1\alpha,25(OH)_2D_3$ was confirmed as a 1α-hydroxylated metabolite by calculating the reduction of the $^3H/^{14}C$ radioactivity ratio[14] (with a change in $^3H/^{14}C$ from 4.9 to 2.7). The *in vitro* synthesis of $1\alpha,25(OH)_2D_3$ by human decidua suggests that in pregnancy the kidney is not the only site of 1α-hydroxylation of 25-OH-D_3. In addition, the present study demonstrates that an extrarenal 24-hydroxylase activity exists in human placenta and decidua.

The findings reported here could be relevant to the understanding of the maternal–fetal interrelationships of vitamin D metabolism. For instance, the placenta could be a major source of the $24,25(OH)_2D_3$ accumulating in the fetus when maternal kidneys are programmed to synthesise $1\alpha,25(OH)_2D_3$. Another possible biological role for the 24-hydroxylase present in the placenta could be the 24-hydroxylation of $1\alpha,25(OH)_2D_3$, in a process similar to that which may occur in the rat intestine[4]. The 24-hydroxylation of $1\alpha,25(OH)_2D_3$, an important step in the inactivation of this metabolite[4,13], could explain the observation that $1\alpha,25(OH)_2D_3$ is undetectable or present only in small amounts in fetal tissues.

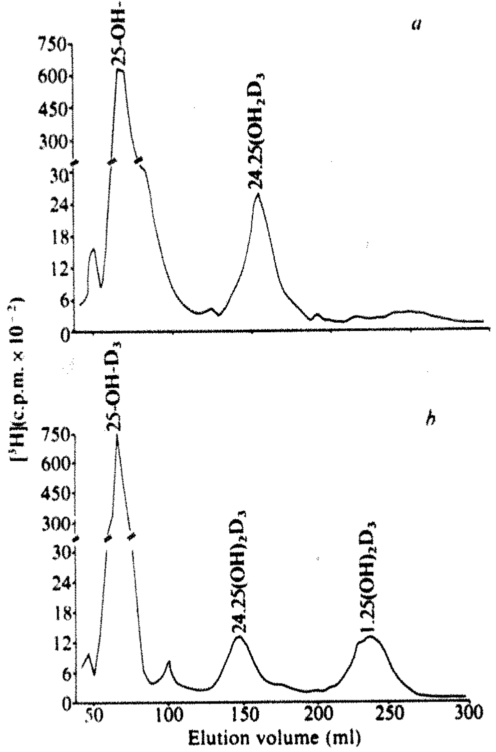

Fig. 1 Characteristic Sephadex LH-20 column chromatographic profile of extracts of full-term human placental tissue (*a*) and full-term human decidual tissue (*b*) incubated with tritiated 25-OH-D_3. Pieces (2 × 2 mm) of the tissues were prepared for culture as previously described[10,11], pooled to an average wet weight of 300 mg and cultured in incubation flasks with 15 ml of RPMI 1640 medium at 37 °C in an atmosphere of 95% air and 5% CO_2 for 48 h, with medium changes every 24 h. At 48 h, 10 pmol $[26,27\text{-}^3H]$25-OH-D_3 (Amersham, specific activity 9 Ci mmol^{-1}) in 0.01 ml ethanol were added to each flask. The tissue and medium were extracted at 72 h with chloroform–methanol[12]. The lipid extracts were chromatographed on a Sephadex-LH 20 column (60 × 1 cm), calibrated with authentic tritiated metabolites and developed with chloroform–petroleum ether (65:35, v/v).

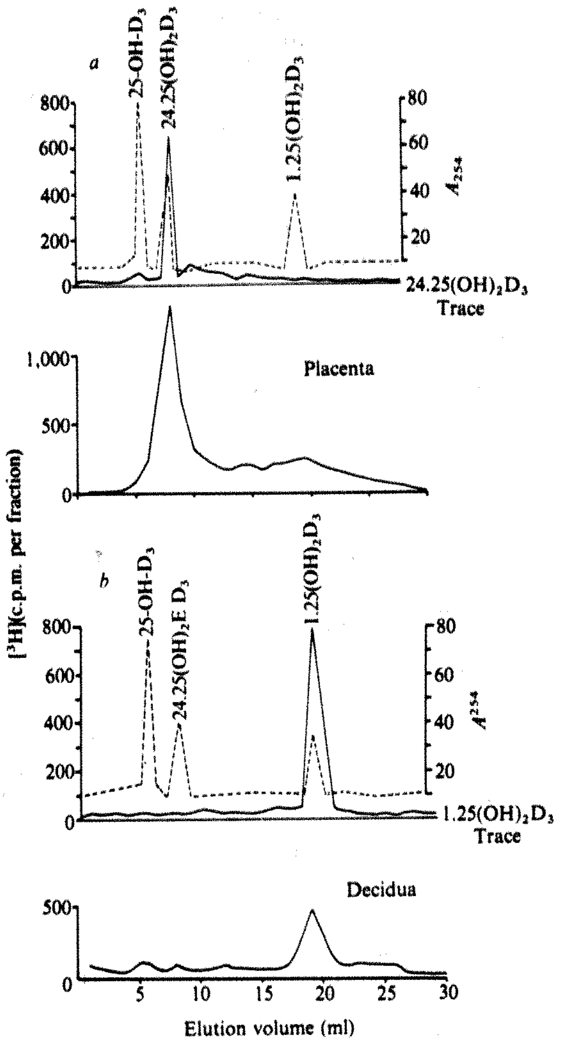

Fig. 2 *a*, High pressure liquid chromatography of the putative $(^3H)24,25(OH)_2D_3$ derived from Sephadex LH-20 columns, synthetic $24R,25(OH)_2D_3$ (----) and biosynthesised ^3H-$24,25(OH)_2D_3$ (——). Extracts of placental tissue incubated for 24 h with $[26,27\text{-}^3H]$25-OH-D_3 were chromatographed initially on Sephadex LH-20 (Fig. 1) and the region corresponding to $24,25(OH)_2D_3$ was then applied to high pressure liquid chromatography (a Waters Associates system fitted with μPorasil columns and eluted with isopropanol–hexane (1:9) with a flow rate of 1.0 ml min^{-1} at a pressure of 280 p.s.i.). A peak of radioactive material coinciding with standard $24,25(OH)_2D_3$ and biosynthesised ^3H-$24,25(OH)_2D_3$ is present. *b*, High pressure liquid chromatography of the putative $(^3H)1\alpha,25(OH)_2D_3$ derived from Sephadex LH-20 columns (Fig. 1*b*), synthetic $1\alpha,25(OH)_2D_3$ (----) and biosynthesised ^3H-$1\alpha,25(OH)_2D_3$ (——). Experimental details are as in *a*. A peak of radioactive material coinciding with standard $1\alpha,25(OH)_2D_3$ and biosynthesised ^3H-$1\alpha,25(OH)_2D_3$ is present.

Similarly, the roles of $1\alpha,25(OH)_2D_3$ synthesised by the decidua remain unclear. One possibility is that it contributes to the observed increase in $1\alpha,25(OH)_2D_3$ levels in the maternal circulation during pregnancy[6]. Alternatively, $1\alpha,25(OH)_2D_3$ synthesised by the decidua may be directly involved in regulating the active placental transport of calcium from the maternal circulation to the fetal compartment. A recent report demonstrating the presence of a placental calcium-binding protein similar to rat intestinal $1\alpha,25(OH)_2D_3$-dependent calcium-binding protein supports this assumption[15].

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The continuity of thick filaments between sarcomeres in honey bee flight muscle

K. Trombitas & A. Tigyi-Sebes

Central Laboratory, Medical University, Pecs, Hungary, 7643

One of the most frequently discussed problems of insect flight muscle morphology is the structure of the thick filaments, especially at the Z line. Many attempts have been made to solve these problems but no unequivocal answers have been given so far. It is well known that physiological specialisation is accompanied by certain anatomical features in the myofibrillar level; the myofibrils have very short I bands in the resting state and the muscle acts under nearly isometric conditions. In this paper, a fresh approach is used to demonstrate the fine structure at the Z line in honey bee flight muscle.

The appearance of the thick filaments and the Z line in insect flight muscles is quite different from that in vertebrate muscles. According to many authors[1-9] there are connections between the thick filaments and the Z line in stretched fibres. In common with all arthropod muscles the Z line consists of a hexagonal network[1,3,10-13] instead of a tetragonal one. The connections between the thick filaments and the Z line were termed 'connecting' filaments[14].

It is very probable that the connecting filaments not only extend to the Z line but also participate in its structure[12]. Consistent with this assumption, it is claimed that there is a continuity of the thick filaments from one sarcomere to the next across the thick filaments prolongations within the Z line[9,13,15,16]. The term 'thick filament' is used here in a structural sense and does not imply that the entire structure contains myosin. On the other hand, no evidence was found for the presence of connecting filaments in unstretched fibres[10,11,17].

Conflicting results have been obtained by electron microscopy of intact muscles using traditional methods (see refs 18 and 19 which present opposite conclusions in the same insect (water bug). Therefore, attempts are now being made to answer these questions using a different technique.

The study of the native isolated filaments can give valuable results for the elucidation of fine structure. Therefore filaments from the honey bee (*Apis mellifera*) flight muscle were isolated according to the method of Morimoto and Harrington[20] with a slight modification. The fresh dorsoventral flight muscles were slightly stretched and soaked in a low ionic strength medium consisting of 5 mM Tris-HC1 and 10 mM dithiothreitol, *p*H 7.8 at 2 °C. After 1 d the muscle was homogenised and centrifuged at 1,000 *g* for 10 min. Then the sediment was resuspended in low ionic strength solution. This procedure was repeated for 7 consecutive days. This period was sufficient to extract the M line material completely and the Z line material partially. Furthermore, an unusual gap or split[21] occurred in the centre of the A band at the level of the former M lines. The cause of the split is unknown, but thick filaments seemed to be completely several in this region.

Finally the myofibrillar mass was centrifuged and resuspended in relaxing medium containing 0.1 M KCl, 5 mM $MgCl_2$, 1 mM EGTA, 5 mM ATP, 20 mM potassium phosphate at *p*H 7.0. This suspension was homogenised three times for 20 s in a Waring blendor. The intact fibrils were centrifuged at 3,000 *g* for 15 min; the turbid supernatant contained isolated filaments. The thick filaments were separated from the thin filaments by zone sedimentation in a density gradient. The supporting solution was the relaxing medium and a linear gradient of glycerol from 10% at the top to 30% at the bottom of the centrifuge tube was used. A 4-ml aliquot of the filament suspension was layered in each tube. This system was centrifuged in a Spinco SW-27 swinging bucket rotor at 22,000 r.p.m. for 2 h. Six equal volume fractions were collected from each tube, and their biochemical composition was studied by SDS-polyacrylamide-gel electrophoresis[22]. The first two fractions contained a high amount of myosin and a low amount of 'unidentfied protein' (perhaps α-actinin, Fig. 1). The third fraction was almost free of protein. The fourth and fifth fractions contained a little myosin, but a high amount of actin. In the sixth fraction distinct and characteristic bands of myosin, paramyosin and actin were found.

A drop of filament solution was applied to a grid coated with carbon film and was negatively stained with 1% uranyl acetate. The first two fractions contained thick filaments. During intense homogenisation, the thick filaments were broken at the split of the former M lines but in every case connections remained at the level of the former Z lines between the very regularly tapered ends of two different thick filaments from opposite sarcomeres (Fig. 2). On several occasions the tapered end of a thick filament bifurcated at the level of the former Z line and joined with two different thick filaments from the adjacent sarcomere (Fig. 3). These intra-Z portions of thick filaments belong to the basic network of the Z line, they pass from one I-Z junction to the next at a slight angle joining the ends of two thick filaments from opposite sarcomeres. This finding correlates with earlier observations of the angling of the Z filaments in longitudinal sections[2,12,23]. Biochemical and structural evidence strongly suggests that this network does not contain actin. It is very probable that the 'unidentified protein' (perhaps α-actinin) can play an important role in the composition of this network, for it

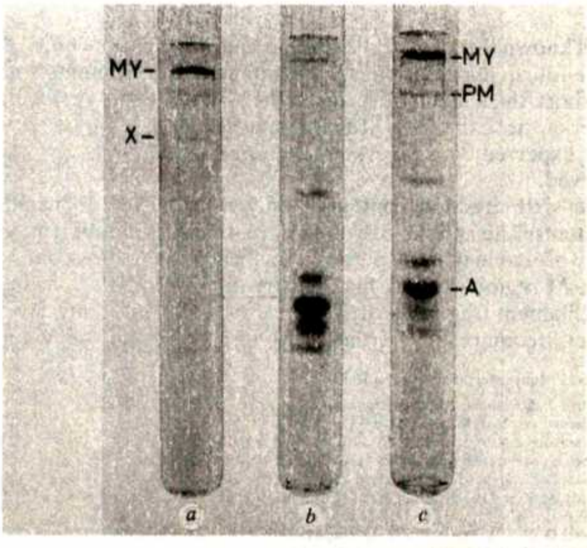

Fig. 1 Gel electrophoresis (10% gel) of sequential fractions obtained from zone sedimentation experiments; (*a*, second fraction; *b*, fourth fraction; *c*, sixth fraction. The bands that were identified by comparison with isolated proteins are marked as follows: A, actin; MY, myosin; PM, paramyosin; X, unidentified protein.

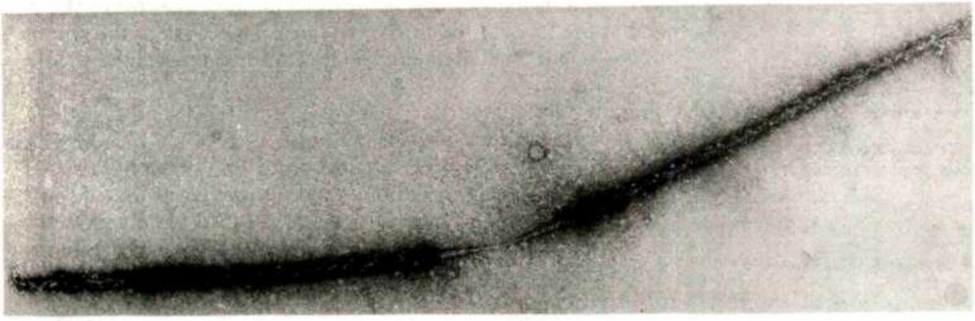

Fig. 2 The very regular tapered ends of two different thick filaments from adjacent sarcomeres, showing them to be connected (×100,000).

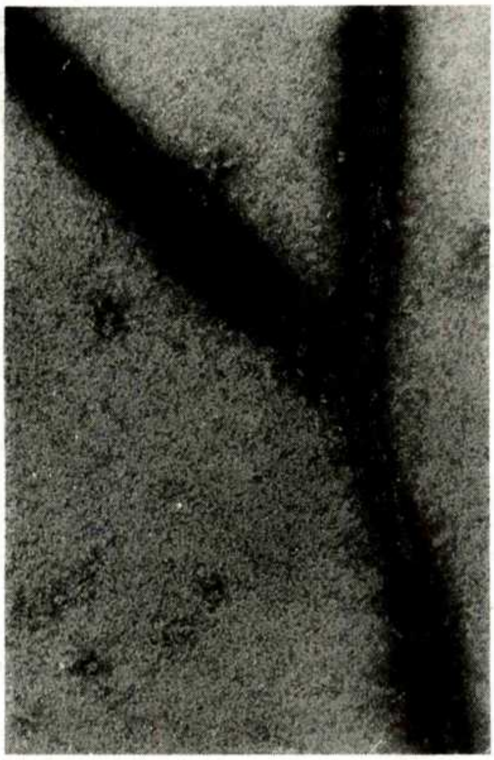

Fig. 3 A single thick filament bifurcated at the level of a Z line and joined with two different thick filaments from the opposite sacromere (×300,000).

is well known that the flight muscle Z line contains α-actinin[21,24].

The fourth and sixth fractions contained thin filaments and a few short thick filament fragments. According to Ashhurst's model of the Z line[10,11,18] thin filaments 1.3 μm long or shorter were expected. However, much longer thin filaments were observed.

The sixth fraction contained only thin and thick filament fragments. The appearance of paramyosin in this fraction makes it very probable that the paramyosin localisation[25–27] is confined to the M region of thick filaments in the bee muscle, since the thick filament fragments originate mainly from the central parts of the sarcomeres where they were disrupted near the M line.

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The stereochemical course of amino acid activation by methionyl- and tyrosyl-tRNA synthetases

Simon P. Langdon & Gordon Lowe

The Dyson Perrins Laboratory, Oxford University, South Parks Road, Oxford, UK

Stereochemical analysis has long been recognised as a powerful tool for elucidating the mechanisms of chemical and enzyme-catalysed reactions. Although much is known about the stereochemical course of reactions at saturated carbon, phosphate and thiophosphate esters whose ligands to phosphorus are also tetrahedrally disposed, are capable in principle of revealing sterochemical information about events at the active site of enzymes that transform such substrates. Nucleotidyl transferases are a group of enzymes which in general selectively use one of the diastereoisomers of a nucleoside 5′(1-thiotriphosphate), such as isomers A and B of adenosine 5′(1-thiotriphosphate), designated ATPαS-A and ATPαS-B, and allow investigation of the stereochemical course of nucleotidyl transfer[1–3]. We have developed a simple method based on ^{31}P nuclear magnetic resonance spectroscopy for determining the stereochemical course of these reactions, and using this method show here that the nucleotidyl transfer step in two aminoacyl-tRNA synthetases from *Escherichia coli* occurs with inversion of configuration at phosphorus. These observations greatly constrain the mechanistic possibilities for these enzymes, and are interpreted most simply as a direct 'in line' transfer from ATP to the amino acid.

Aminoacyl-tRNA synthetases catalyse two distinct reactions, namely, amino acid activation by MgATP with formation of a mixed anhydride between the amino acid and AMP, and the transfer of the aminoacyl moiety of the aminoacyladenylate so formed to the cognate tRNA[4].

$$\mathrm{H_3\overset{+}{N}{\cdot}CHR{\cdot}CO_2^- + MgATP \rightleftarrows H_3\overset{+}{N}{\cdot}CHR{\cdot}CO{\cdot}AMP + MgPP_i}$$

$$\mathrm{H_3\overset{+}{N}{\cdot}CHR{\cdot}CO{\cdot}AMP + tRNA \rightleftarrows H_3\overset{+}{N}{\cdot}CHR{\cdot}CO{\cdot}tRNA + AMP}$$

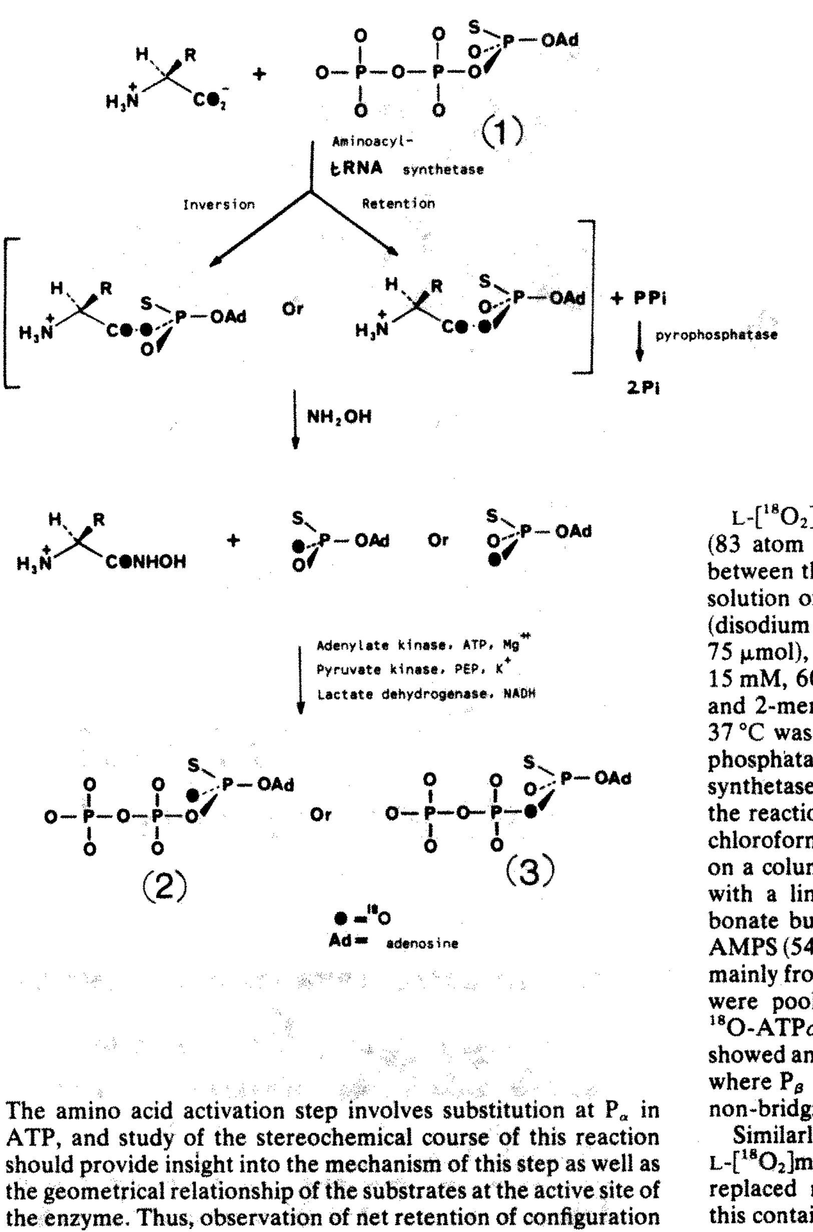
(1)
Aminoacyl-tRNA synthetase
Inversion
Retention
Or
+ PPi
pyrophosphatase
2Pi
NH$_2$OH
Or
Adenylate kinase, ATP, Mg^{++}
Pyruvate kinase, PEP, K$^+$
Lactate dehydrogenase, NADH
(2)
Or
(3)
● = ^{18}O
Ad = adenosine

Fig. 1 In an aminoacyl t-RNA synthetase-catalysed reaction between the cognate [$^{18}O_2$]-amino acid and ATPαS-A (1) in the presence of hydroxylamine, inversion of configuration at P_α leads to adenosine 5'[S-^{18}O]phosphorothioate and retention of configuration leads to adenosine 5'[R-^{18}O]phosphorothioate. These enantiomeric ^{18}O-phosphorothioates can be distinguished after conversion enzymatically to ATPαS-A (refs 6, 7), by determining whether ^{18}O is in the non-bridging (2) or bridging position (3), using ^{31}P NMR spectroscopy.

The amino acid activation step involves substitution at P_α in ATP, and study of the stereochemical course of this reaction should provide insight into the mechanism of this step as well as the geometrical relationship of the substrates at the active site of the enzyme. Thus, observation of net retention of configuration would be consistent with the intermediary of a nucleotidyl enzyme whose formation and breakdown follow the same stereochemical course, or an adjacent attack by the amino acid at P_α of ATP, followed by a single pseudorotation. Alternatively, net inversion of configuration would be interpreted most simply as a direct 'in-line' displacement.

If ATPαS-A (1) (Fig. 1), which is now known to possess the S-configuration at P_α (refs 3, 5), is an acceptable substrate for an aminoacyl-tRNA synthetase, incubation in the presence of the cognate [$^{18}O_2$]-amino acid and a high concentration of hydroxylamine should lead to the irreversible formation of the amino ^{18}O-hydroxamic acid and adenosine 5'[^{18}O]phosphorothioate (^{18}O-AMPS). If nucleotidyl transfer occurs with inversion of configuration at P_α, the *pro-S* oxygen would be labelled, whereas retention of configuration at P_α would place ^{18}O in the *pro-R* position (Fig. 1). Conversion of adenosine 5'-phosphorothioate (AMPS) into ATPαS-A by the combined action of adenylate kinase and pyruvate kinase involves phosphorylation of the *pro-R* oxygen[3,5]. Thus, if ^{18}O is present in the *pro-S* position in ^{18}O-AMPS, ^{18}O will be found in the non-bridging position at P_α in ATPαS-A (2) (Fig. 1), whereas if ^{18}O is present in the *pro-R* position in ^{18}O-AMPS, it will be found in the P_α–O–P_β bridge in ATPαS-A (3) (Fig. 1). These alternative possibilities can be differentiated by ^{31}P NMR spectroscopy[5], as ^{18}O in the P_α–O–P_β bridge produces an isotope shift on both P_α and P_β, whereas ^{18}O in the non-bridging position causes an isotope shift at P_α only.

L-[$^{18}O_2$]methionine (88 atom % ^{18}O) and L-[$^{18}O_2$]tyrosine (83 atom % ^{18}O) were prepared by acid-catalysed exchange between the L-amino acid and [^{18}O]-H_2O (99 atom % ^{18}O). A solution of L-[$^{18}O_2$]methionine (16 mg, 110 µmol), ATPαS-A (disodium salt, 42 mg, 75 µmol), magnesium acetate (17 mg, 75 µmol), Tris (242 mg, 2 mmol), hydroxylamine solution (4 ml, 15 mM, 60 mmol), phenylmethanesulphonyl fluoride (100 mg) and 2-mercaptoethanol (0.2 ml) in deaerated water (60 ml) at 37 °C was adjusted to *p*H 8.0 with 1 M HCl. Inorganic pyrophosphatase (0.5 mg) was added, followed by methionyl-tRNA synthetase (4 mg). The solution was kept at 37 °C for 48 h and the reaction terminated by addition of EDTA (120 µmol) and chloroform (10 ml). The aqueous phase was chromatographed on a column of DEAE-Sephadex A-25 (HCO_3^- form) at 4 °C, with a linear gradient of aqueous triethylammonium bicarbonate buffer (0.2–0.7 M, *p*H 8.0). Fractions containing ^{18}O-AMPS (54 µmol, this contained some unlabelled AMPS derived mainly from the hydrolysis of ATPαS-A during the incubation) were pooled and the buffer removed. It was converted to ^{18}O-ATPαS-A enzymatically[6,7]. P_α of the ^{18}O-ATPαS-A showed an isotope shift of 1.1 Hz (at 36.43 MHz) to higher field, where P_β showed none, demonstrating that the ^{18}O was in the non-bridging position at P_α.

Similarly, when L-[$^{18}O_2$]tyrosine (23 mg, 110 µmol) replaced L-[$^{18}O_2$]methionine and tyrosyl-tRNA synthetase (4 mg) replaced methionyl-tRNA synthetase, ^{18}O-AMPS (43 µmol, this contained some unlabelled AMPS derived mainly from the hydrolysis of ATPαS-A during the incubation) was obtained and converted enzymatically[6,7] into ^{18}O-ATPαS-A. Again, P_α showed an isotope shift of 1.1 Hz (at 36.43 MHz) whereas P_β was unaffected, demonstrating that the ^{18}O was in the non-bridging position at P_α.

These observations show that the nucleotidyl transfer step catalysed by both methionyl- and tyrosyl-tRNA synthetase from *E. coli* occur with inversion of configuration at phosphorus.

The simplest interpretation of these results is that nucleotidyl transer catalysed by these enzymes occurs by an in-line mechanism at P_α. Although more complex multi-step pathways (for example, three steps, each with inversion of configuration, or an adjacent attack with permutational isomerism) cannot be rigorously excluded, it seems unlikely that such mechanisms could occur in the confines of the active site of an enzyme.

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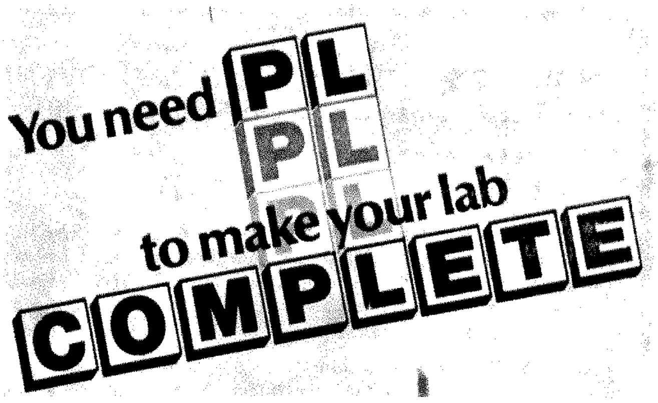

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reviews

Survey of medical history

Sydney Selwyn

Explorers of the Body. By Steven Lehrer. Pp. 463. (Doubleday: New York, 1979.) $12.95.

POPULAR accounts of medical history are surprisingly scarce. Before the appearance of Dr Lehrer's book, the only notable example of this rare genre was *Microbe Hunters*, which was first published over 50 years ago. The author, Paul de Kruif, was a lapsed bacteriologist whose highly coloured descriptions of heroic battles against infection fired the imagination of a wide audience. Indeed, many impressionable adolescents were inspired by his book to enter medicine and related professions.

Sadly, de Kruif's sequels on nutrition, psychiatry and other medical themes were progressively — perhaps predictably — less successful; and although he can be regarded as the main originator of the racy, home-spun style of medical journalism, which remains so popular in North America, it is puzzling that so few writers have attempted to emulate him with full length work in one of the most fascinating branches of human history. Since the 1920s an intermittent series of unexciting biographies has only occasionally been relieved by something as imaginative as *Rats, Lice and History*, written by another bacteriologist, Hans Zinsser. So barren and featureless has been the landscape, that this whimsical little book of 1935 still remains outstanding.

The publication of a reasonably comprehensive survey of medical history for the general reader is therefore to be cordially welcomed. Written attractively and with authority by a practising physician, this new book builds up a mosaic of the lives and work of innumerable contributors to the development of medical science. Four central chapters out of ten are devoted to infection and its control, the remainder being idiosyncratically arranged, starting with physiology, moving to genetics and then anaesthesia. Surgery is embedded among the infections, and the book ends with X-rays and diabetes mellitus.

The opening chapter on the evolution of knowledge about the functioning of the body forms a very satisfying prelude to this vast epic. From the stumbling observations made in Ancient Greece and Rome on the action of the heart and on respiration, progress is clearly traced through the late Renaissance to the work of William Harvey and a succession of other pioneers. Many of them were not primarily interested in medicine, for example, Boyle, Hooke, Priestley and Black in Britain, and Lavoisier and Laplace in pre-revolutionary France. As an incidental feature, the often uncomfortable (and occasionally fatal) effects of politics on the lives of medical explorers are curiously evident in this introductory section.

Similarly, in the two subsequent chapters fresh insight is provided into such well rehearsed stories as the work of Mendel, the elucidation of the structure of DNA, the acrimonious and complex background to the introduction of anaesthetic gases, and the rise and fall of mesmerism. Although inevitably told with less verve than in de Kruif's pages, Dr Lehrer's accounts of microbe hunting and taming make compelling reading. Particularly vivid are his descriptions of vaccines, antimicrobial drugs and, indeed most other medical advances.

A special feature of the book is its very revealing portraiture of major and minor participants alike, While most are necessarily depicted with the economy of a miniaturist, so extensive yet compact a gallery is unprecedented. Even the cognoscenti will probably realise for the first time just how disagreeable the average medical pioneer is. For every saintly figure such as William Withering or Paul Ehrlich, the author delineates dozens of irascible, bombastic, unscrupulous, or at the least, devious individuals such as Paracelsus, Hooke, Priestley, Erasmus Darwin, Semmelweis, Mendel, Pasteur, Koch, Cushing, Behring, Ross, and a handful of more recent Nobel laureates.

Sometimes, however, debunking seems to be indulged in for its own sake. For example, Harvey is said to have "lacked the ability of a skilled experimenter" because he considerably underestimated the body's total blood volume (which even now is difficult to measure). Edward Jenner is also criticised on the rather tenuous grounds that he did not publish details about the death of one of his patients, who seems to have succumbed to an unrelated "infection". A further criticism is that Jenner regarded cowpox and a comparable infection of horses as being closely related both to one another and to human smallpox — which indeed is in accord with modern virological views. But after the uncritical recent apotheosis of Harvey during the celebrations of his 400th anniversary, and the sustained adulation of Jenner during the past 150 years, fair re-evaluation of their work is very welcome. The text contains relatively few typographical or factual errors, and solecisms such as the statement that the French Academy of Sciences was, in the eighteenth century, "the final authority in European medicine" are mercifully rare.

The reader may well be disappointed to find virtually no mention of pathology, pharmacology and several medical specialties, including psychiatry — the "Explorers of the Body" being zealously prevented from entering the domain of the mind. An account of some of these important subjects could well have replaced the largely non-medical section on genetics. Nevertheless, the author has accomplished a *tour de force*, and his publishers also deserve praise for producing this admirable book at so reasonable a price. ☐

Sydney Selwyn is Reader in Medical Microbiology at the Westminster Medical School, University of London, UK.

What world food crisis?

Nutrition and the World Food Crisis. By Mary Alice Caliendo Pp.368. (Collier-Macmillan: London, 1979.) £5.95.

ONE function of the applied scientist is to warn his fellow man of possible impending doom, but there is a very real danger of loss of credibility if "Wolf!" is cried too often. There has been a spate of books forecasting food shortage and mass starvation over the past fifty years (two hundred if one counts Malthus), but although the situation is bad it has not noticeably become worse: food production per head of population has remained almost constant. Indeed, there has been a growing realisation that there is more than enough food about and that the real problem is that the poor cannot afford to buy their daily bread.

Food requirements are about 2,000 kilocalories per day per man, woman and child, and global agriculture produces something in excess of 4,500 kilocalories per head per day of crops suitable for human consumption. Much of this food is used inefficiently for the feeding of livestock to provide animal products for the rich, but even so there is about 2,500 kilocalories per head per day available for human consumption. So, please let us not hear about the 'World Food Crisis'.

All the evidence suggests that farmers can cope with the supply problem providing it is made worth their while. Malnutrition is not a supply problem: essentially economic demand does not equal physiological need. Very roughly half the world is underfed and the other half overfed; and it can be shown that the diseases of affluence — for example, obesity, heart disease, dental caries — are numerically of the same order as those associated with undernutrition. This poor distribution of wealth between nations is of course exacerbated by a further poor distribution within nations, which can lead to local famines. But even in the oft cited Ethiopian famine there was no shortage of food in the country as a whole: the rains failed in one area and the subsistence farmers there did not have enough money to buy food from the market in Addis Ababa.

Nevertheless, the International Agencies constantly document the wide extent of malnutrition in the world and proffer much advice on what to do about it. Not surprisingly this advice is not always consistent: experts often see things from their own narrow viewpoint, and generalists who can grasp technical, economic, social, cultural and political aspects of a problem are rare and often under-rated. Dr Caliendo has made a bold attempt to gather the relevant material, especially that since the 1974 World Food Conference. But sadly the inconsistencies have not been analysed critically or even pointed out. The need to increase food production is stressed in one chapter, but the next on distribution begins with the statement that production is about twice that needed. In chapter 2 the misplaced emphasis in the past on the role of protein in the aetiology of malnutrition is revealed, but in chapter 12 protein-rich foods are promoted. The importance of socio-economic factors is stressed in chapter 10 but their relevance to infant feeding practices in chapter 9 is not adequately discussed: bottle feeding is adopted by many intelligent mothers because it enables them to go out to work and thus improve the economic (and nutritional) status of their families. The current fashion for national food and nutrition planning is outlined but the fact that developed countries eliminated undernutrition without it is not mentioned, nor are we given one example that it has worked anywhere. Food aid is documented without definition nor mention that it is rarely food and seldom used to feed hungry people. These few examples are a true reflection of the inconsistency of the documents available, but perhaps it would be strange if such a complex problem as world hunger were to have a single simple solution.

Many modern texts are collections of papers by various authors often from Symposia and have the disadvantage that the information is not integrated as in good old-fashioned textbooks. Dr Caliendo has sadly not grasped the opportunity to do this but tries to be all things to all men. It is difficult to see where she stands herself. The book is aimed to "provide educators and students with substantive and practical information" with a view to "make it easier to learn about the problems of global hunger and nutrition". There is no doubt that it is a most useful compendium of information but because it is so uncritical I suspect it will confuse all but the brightest student. However, the book is cheap, extremely well illustrated with photographs, tables and graphs, and a jolly good read.

D.S. Miller

D.S. Miller is a Research Fellow in the Department of Nutrition at Queen Elizabeth College, University of London, UK.

Einstein's influence on politics and philosophy

Albert Einstein: His Influence on Physics, Philosophy and Politics. Edited by P. C. Aichelberg and R. U. Sexl. Pp. 220. (Friedr. Vieweg: Wiesbaden, Germany, 1979.) DM48.

I NEVER really liked C.P. Snow's theory of the Two Cultures. In the United States of the 1950s and 1960s it seemed to me that scientists had a good grasp of politics and that liberal arts graduates were genuinely interested in and capable of understanding the main lines of scientific development. But perhaps the only way to understand the durability of the myth of Einstein as a socially naive genius is to accept Snow's basic assertion — there exist two cultures, literary and scientific, whose members cannot talk to each other. On the science side, Einstein's physics continues to mystify the general educated public. And on the arts side Einstein's politics remain incomprehensible to scientists who invariably characterise him as being naive when they write about his life.

The present volume of essays, commissioned by the International Society on General Relativity to celebrate Einstein's centenary, misses its chance to balance the picture. Editors Aichelberg and Sexl state in their introduction that the collection demonstrates the "far reaching influence of Einstein's work and his personality on physics, philosophy, theory of science and on the politics of our time". I believe that such a demonstration is possible. But this collection fails in its attempt to present a comprehensive view of Einstein's influence because much of the physics reported will be understood only by specialists and because Einstein's politics and philosophy receive the usual banal characterisation. The editors re-express the old cliché. Einstein's philosophical directness is "naive" and "this naivite is also characteristic for Einstein's involvement towards politics in which he was increasingly involved in his later years". The summary is wrong on both counts and it is time to do better.

Einstein was involved in politics from at least 1914 onwards and his political actions throughout his life reflected a highly sophisticated socialist's understanding of what was possible for a man in his position to do at any time. Beginning with the courageous anti-War manifesto in Berlin in 1914 which attacked the extreme national chauvinism of War-time Germany, and ending with his support for Julius and Ethel Rosenberg executed in the US for conspiracy to commit treason in the McCarthyite hysteria of the 1950s, Einstein intervened consistently against militarism, racism and fascism. And though his actions have seemed quixotic to close colleagues such as Max Born, they were informed by a detailed socialist analysis of the workings of class society.

It is possible to indicate only briefly the depth of Einstein's approach. Here is his summary of an analysis called "Why Socialism" that he wrote in 1949 for the influential US magazine, *Monthly Review:*

> "The situation prevailing in an economy based on the private ownership of capital is thus characterised by two main principles: first, means of production (capital) are privately owned and the owners dispose of them as they see fit; second, the labour contract is free. Of course there is no such thing as a *pure* capitalist society in this sense. In particular it should be noted that the workers, through long and bitter political struggles have succeeded in securing a somewhat improved form of the 'free labour contract' for certain categories of workers. But taken as a whole, the present day economy does not differ much from 'pure' capitalism".

Einstein was clear about the solution to the problems that this social arrangement causes:

> "I am convinced that there is only *one* way to eliminate these grave evils, namely through the establishment of a socialist economy accompanied by an educational system which would be oriented towards social goals".

These are not the views of a neophyte. Far from being naive, Einstein's radicalism contrasts favourably with the results achieved by the inept liberal nationalism of the German scientific elite. Professor Carl-Friedrich von Weizsäcker's piece on Einstein's politics illustrates the gap between the approaches. For von Weizäcker it was Einstein's "aloofness" not his greater political understanding that permitted Einstein to act politically while others, as von Weizsäcker puts it, "conceded — apparently or truly — to believe in the political prejudices of the time in order to function concretely in their midst".

Similarly Banesh Hoffman presents only a superficial view of Einstein's Zionism by failing to situate Einstein's support for the state of Israel in the context of the intense anti-semitism of Europe between the Wars. As Einstein said in 1938:

> "Rarely since the conquest of Jerusalem by Titus has the Jewish community experienced a period of greater oppression than prevails at the present time".

Einstein supported the Zionist cause pragmatically and I think it is a mistake to present him as a narrow nationalist. This was his appraisal of the situation in 1930:

> "I saw how schools, comic papers and innumerable other forces of the Gentile majority undermined the confidence of even the best of my fellow-Jews and felt that this could not be allowed to continue. Then I realised that only a common enterprise dear to the heart of Jews all over the world could restore this people to health".

But in spite of the fact that the collection does not live up to the promise of its title there is still much to interest the casual reader. Dennis Sciama offers a nice concise review of the current position in cosmology and Roger Penrose delivers an informative and very nearly comprehensible account of black holes. And of continuing interest are the personal reminiscences of Einstein. Walther Gerlach on Einstein and quantum theory and John Wheeler on Einstein's Princeton days are new additions to the Einstein archive. Particularly welcome is the inclusion of Einstein's last lecture which along with the transcript of his answers to the questions give a vivid picture of Einstein in action as a thinker. Finally, Wolfgang Yourgrau's portrait, "Einstein — and the Vanity of Academia" is a fresh and irreverant view of the German academy in the 1920s. Yourgrau may have gone too far in reporting his mother's shocked observation that Einstein had dandruff, but I think Einstein would approve. The idea of a god with dandruff would have amused him immensely.

Joe Schwartz

Joe Schwartz is on leave from the City University of New York.

Communicative abilities

Studies of Language Thought and Verbal Communication. Edited by R. Rommetveit and R. Blakar. Pp. 466. (Academic: London and New York, 1979.) £22.80.

OVER the past 20 years Rommetveit has been developing a particular approach to the psychology of language, thought and communication. He calls his position a "social-cognitive" one and it contrasts in particular with the psycholinguistic-experimental approach with its focus on single sentences. Rommetveit has always held that such an approach has led to gross simplifications which could never be of use in comprehending the complexities of the mechanisms underlying our communicative abilities.

This book is a collection of articles by Rommetveit and his collaborators in Oslo. It is a mixture of theoretical statements and experimental articles. The 35 articles are of very mixed value and there is little attempt to link together the varied work which ranges from word recognition to the verbal maintenance of sex rules in Norwegian. The experimental section of 27 articles would have benefited from severe pruning.

There is better value in the theoretical articles (most of which are not easily available in their original places) where Rommetveit draws on a number of linguistic and philosophical traditions in discussing the nature of language and communication and moving over into hermeneutics and social psychology. I didn't always succeed in understanding, and quite frequently disagreed but found myself stimulated agreeably often. However, Rommetveit never achieves the synthesis he aims for.

John Morton

John Morton is a member of the scientific staff of the MRC Applied Psychology Unit, Cambridge, UK.

Vibrations of molecular crystals

Molecular Vibrations in Crystals. By J.C. Decius and R.M. Hexter. Pp. 391 (McGraw-Hill: New York and London, 1979.) £15.60; $29.50.

MANY textbooks discuss in considerable detail the lattice vibrations of simple covalent and ionic crystals but this book is unique in dealing with the vibrations of molecular crystals. As these are of the type that most chemical solid-state spectroscopists deal with, this book will be of interest and value to the growing number of scientists who study experimentally the vibrations of molecular crystals using infrared, Raman or neutron spectroscopy or to theoreticians carrying out lattice dynamical calculations on molecular crystals. It is jointly written by two authors with an international reputation for their contributions to the vibrational spectra of molecular crystals using infrared spectroscopy. In many ways the book is a natural extension of the now classic work on *Molecular Vibrations* by Wilson, Decius and Cross (McGraw-Hill) which deals with isolated molecules.

The original approach used in this book is to emphasise a representation based on internal coordinates and thus to take advantage of molecular integrity where it is appropriate, but to permit its modification by perturbation theory. In the internal coordinate representation the dynamical matrix becomes the familiar FG matrix of Wilson, Decius and Cross. A new method of calculation of this matrix, valid throughout the Brillouin zone is developed in this treatise.

Many chemical spectroscopists are not familiar with the splitting of longitudinal and transverse optical modes; they will therefore find useful the review of this important topic which is treated both from the traditional macro-scopic point of view as well as with microscopic theory. This has not previously been so carefully done.

Six different molecular crystals (HCN, CO_2, CHI_3, NaN_3 and $NaClO_3$) are used as detailed examples to consider infrared and Raman spectra and their selection rules. In all cases, spectra are discussed with respect to crystal splittings and polarisations. The current theories of these splittings are carefully examined, compared and evaluated in molecular as well as in ionic crystals. The expression of intermolecular coupling of vibration in both excitation and phonon coordinates is contrasted, and the basis and extent of their equivalence is discussed.

The theory of multiphonon spectra is thoroughly reviewed, covering selection rules, intensity distribution and origin in crystal anharmonicity. The Van Hove and Phillips theories of critical points are carefully explained. In one example, Iodoform-calculated phonon dispersion curves are presented and their consequences are compared with experiment.

In the final chapter, the spectra of impure crystals, both ionic and molecular, are discussed, together with a simple introduction to Green's function treatment of isotopically impure crystals.

The authors are to be congratulated on producing a well written definitive work that will appeal to physicists as well as chemists interested in molecular crystals. This book is likely to prove useful as a text for postgraduate courses on infrared and Raman spectra of solids, self-study reading for students starting research in the subject and as a guide to the extensive literature in this field.

G.R. Wilkinson

G.R. Wilkinson is Professor of Physics at King's College, University of London, UK.

Psychosocial evolution

Sir, — In his review of Garland Allen's *T.H. Morgan: The Man and His Science* (see *Nature*, **278**, 786-787; 1979), Professor Darlington makes several very cogent points. Perhaps the most illuminating of all are his remarks about the relations between Morgan, Wilson and Bateson, which are especially valuable in view of the fact that he knew all three men. It is therefore a great pity that, simply for the sake of taking a rather poorly aimed pot-shot at Allen's Marxism, Professor Darlington drags in the *canard* of "Lamarckism and Lysenkoism", alleging that both Morgan and Julian Huxley opened the door to these horrors. I do not have Morgan's *Scientific Basis of Evolution* to hand but what Huxley says on psychosocial evolution in *Evolution: The Modern Synthesis* (Allen and Unwin: London; second edition, 1963, pp. xliv - xlvii) in no way justifies this charge. It might be objected that "psychosocial evolution" is a cumbrous and pretentious phrase for a very simple and obvious phenomenon. Indeed, I would myself prefer simply "cultural evolution", or the phrase that Huxley used elsewhere in a popular essay (*The Uniqueness of Man*; Allen and Unwin: London; 1941): "cumulative tradition". The idea is a commonplace, for which a list of 'sources' would be neither necessary nor practicable. This is itself sufficient reason for Huxley not having mentioned Morgan in this connexion. To argue that, because of its radically different nature, it should not be regarded as a form of evolution or of heredity would be difficult in view of the dictionary meanings of those words.

A belief in psychosocial evolution is perfectly compatible with the belief that heredity, in the genetical sense, is diamond-hard. For Professor Darlington to pretend otherwise is just as absurd as it is for Arthur Koestler (*The Case of the Midwife Toad*; Hutchinson; London, 1971) to claim that Waddington's work on genetic assimilation was a vindication of Lamarckism. Nobody who approaches either human history or what is going on in the world today with a relatively unprejudiced mind can doubt that psychosocial evolution is both real and important. To create a false link with Lamarckism is merely to hand the argument to the Lamarckians on a plate.

A.G. Cock

A.G. Cock is Lecturer in the Department of Biology, at the University of Southampton, UK.

Cock on evolution

Dr Cock complains that I have wrongly connected the evolutionary views of Morgan and Huxley. And also wrongly connected them with the theories of Lamarck and Lysenko and with what I called the "froth" of our social sciences today. These ideas, he says, are "commonplace" and it is not "necessary or practicable" to trace them to their roots. I, however, think it necessary to trace all ideas, scientific or political, true or false, to their roots. I have been trying to do so for over fifty years. May I show how it works?

There were two roots in this case. Morgan had used his reputation as the supposed creator of the theory of heredity to advance a false theory of heredity and evolution in man. And Julian Huxley had used his name to repudiate his grandfather's work in *Man's Place in Nature;* he was therefore able to present himself as the Darwinian who had discovered that after all we need not worry: Darwinism did not apply to man.

Take Morgan first. I did not seriously look at his book when he gave it me in 1933 (I don't think he looked at my book either). But there it stands. It expresses the main fallacies current in human evolutionary thought today. The first fallacy is that "There are in man two processes of inheritance: one through the physical continuity of the germ-cells; and the other through the transmission of the experiences of one generation to the next". The physical inheritance gives you 3:1 ratios and dogmatic truth of interpretation. It applies to bodily characteristics. But (and here is the second fallacy) it does not apply to the brain: "It is the plasticity of man's brain that makes him unique". Then Morgan naturally asks: "Is intelligence inherited?". How can we be sure? There is nature and nurture. And there may be conflict between them. Worse still, there is "the doctrine that all men are born free and equal". All the issues are avoided. All the fallacies are scrambled.

Three years later (in 1936) this kind of loose talk had become dangerous. In Europe genetic assumptions were being turned to political uses. Half-baked genetic ideas were helping us on the way to war. In this country a particular danger concerned me. Genetic teaching and research hardly existed in our universities. I therefore collected university signatures for a letter in *Nature* (**138**, 972-973; 1936) protesting against academic stagnation in this vital respect. I also turned to Huxley who had been aggressively defending the Mendelian and Darwinian positions at the British Association. I persuaded him to edit an international symposium on the developing situation in evolutionary theory. He agreed. But by the time the book appeared in 1940 (under the title of *The New Systematics*) I discovered that Huxley was easing out of the genetic problem so far as man was concerned. In *We Europeans*, followed by the *Uniqueness of Man* he was looking for a political way out: the kind of 'placebo' that Morgan had stumbled upon.

Faced with this confusion I tried to make my position clear in an article, or manifesto, under the heading of "Race, Class and Mating in the Evolution of Man" (*Nature*, **152**, 315-319; 1943). Here I discussed the complex and often reciprocal or feedback relations of race, culture and selection, as recorded in history but usually misunderstood by historians. I argued that inbreeding always produces groups or communities or races which are the indispensible agents of human evolution.

Each of these "owes its character to what we may call its chromosome pool". I went on to say that "race made culture and language" and that inbreeding led to "easy transmission of culture". In other words the creation and transmission of ideas were both based on hereditary abilities, and both were subject to genetic and selective Mendelian and Darwinian processes.

To justify these *ex-cathedra* statements I began to set out the historic and pre-historic record of human society in genetic terms. My first essay, *The Facts of Life*, I sent to Huxley in proof, as he had sent me the chapter of his *Evolution* dealing with my work. He made prodigious annotations, chiefly urging me to qualify my generalisations or weaken my conclusions. I kept the proof but did not adopt the suggestions.

During the next ten years we exchanged a hundred or so letters in which Huxley was enquiring about my views on genetic and evolutionary questions. At the end of this time (in 1963) he published a second edition of his *Evolution*, with a new introduction. Here he set out his full UNESCO formula. Now all peoples were to be reconciled to one another, prosperously united under the banner of inevitable scientific progress.

Man had entered a "psychosocial phase of evolution". Change was now "primarily cultural and only secondarily genetic". Each human population had, not my pool of chromosomes, but "a pool of ideas" on which it could draw "for its evolutionary requirements". It was obvious (as I explained in the *Times Literary Supplement* of 20 December, 1974) that in this pastiche Huxley had provided the world with a pool of ideas which would meet the "evolutionary requirements" of the social and political sciences for many years to come. Not that they would know (any more than Dr Cock does) what pool they were drawing them from.

For there was indeed another pool. The Soviet Government had repudiated Lysenko in agriculture. But Marxists throughout the world continued to draw what unity they had from one surviving belief, the belief that theories of evolution by natural selection did not apply to man. Anathema had been pronounced on 'Social Darwinism' and it could never be withdrawn. So abominable was this heresy that it must always be condemmed; and it must never be discussed.

For these reasons I thought it worthwhile to write another book, *The Little Universe of Man* (apparently unknown to Dr Cock but reviewed in *Nature*, **277**, 247; 1979). Here I argued in elementary terms my genetic view that intelligence is seated in the brian and that the brain is part of the body. Hence men differed in their ability to create culture, to acquire culture and to transmit culture. Not only men and women as individuals, but as families, classes and races, as sects and groups of all kinds, they differed genetically by their genes and chromosomes in their abilities to do these things. They always had differed and always would differ, a situation responsible for the past history, present state and future prospects of mankind.

In writing these things I took the opportunity again of holding up Huxley's views to the ridicule they invite and deserve. I also again returned to expose a minor fallacy of Morgan's which has left a long trail of error behind it. This is the view that all heredity is in the chromosomes and in the nucleus. And that therefore one-egg twins are always identical in heredity. Each step in this argument is false and the conclusion has been repeatedly falsified. But it had become, as I put it (*Nature* **234**, 521-525; 1971) "*axiomatic*". Because Morgan said it (although he also said intelligence could not be measured) all psychologists trying to measure intelligence have assumed it to be true — and that includes the late Cyril Burt. I therefore pointed out, first, that Galton, who first recognised one-egg twins, never made Morgan's mistake of supposing they were identical; and secondly, that there are two genetic ways, nuclear and non-nuclear, in which we know why they often cannot be identical. The likenesses of one-egg twins, whether measured in mind or body, remarkable as they are, thus always give an under-estimate of the effects of heredity; their differences always give an over-estimate of the effects of environment.

I may now go back to Dr Cock and his sources. I have found that whether in scientific, historical or any other discussion, it is always desirable to look for the sources, examine them, and question them; and each step in the argument that has led from them. And I believe it is because social and political scientists often fail to do so, finding that the knowledge is "commonplace", or the task not within their "frame of reference", or merely, following the eminent examples of Morgan and Huxley, that so much of what flows from their enquiries turns out to be froth.

C.D. Darlington

C.D. Darlington is Emeritus Professor of Botany at the University of Oxford, UK.

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3280(A)

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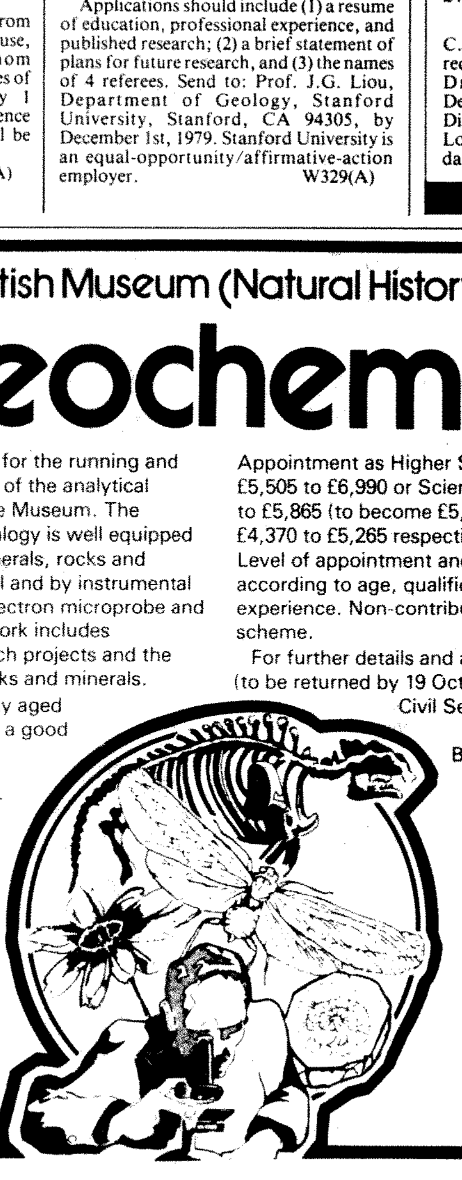

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Applicants should have high academic qualifications and experience in a field or fields relevant to the subject and have a proven record in research and research management. Salary will be:

CHIEF OF DIVISION, GRADE 1 $A32412

Application forms may be obtained by writing to the Counsellor (Atomic Energy), Australian High Commission, Australia House, Strand, London, WC2B 4LA, with whom applications will close on 12 October 1979.

3312(A)

DEPARTMENT OF AGRICULTURE FOR NORTHERN IRELAND

HEAD OF POULTRY DEPARTMENT
£7,350 — £8,722

Applications are invited for the pensionable post of Head of Poultry Department at Loughry College of Agriculture and Food Technology, Cookstown, County Tyrone.

The College offers Certificate courses in Agriculture, Ordinary and Higher National Diploma Courses in Food Technology and a Higher National Diploma in Poultry Husbandry. In association with Queen's University, Belfast, 3 courses at post-graduate level are provided. Two of these lead to the degree of MAgr (by examination) and are offered in Dairy Technology and Poultry Technology. There is also a course in Agricultural Communication which leads to the award of a University Diploma. A variety of other courses to meet the needs of the food production and processing industries is given as the need arises.

DUTIES

The duties of the Head of Department are:

(a) Organisation and supervision of all teaching within the Department.

(b) Organisation and supervision of the Poultry production and processing enterprises at the College.

(c) Organisation of the participation in applied research in poultry.

(d) Liaison with the Poultry Advisory Services of the Department of Agriculture, Northern Ireland in the provision of advice to poultry producers and processors.

Some additional supervisory and stand-by duties are involved. Research work may be submitted to the University for higher degrees. A modern residence is available if required.

QUALIFICATIONS

Candidates must have a first or upper second class Honours Degree or a post graduate degree considered to be of comparable value. The degree should be in Agriculture, Agricultural Science or Science or in a subject area which relates to the science and practice of poultry production and marketing. Extensive experience in teaching or research in poultry or a closely-related field is essential.

SALARY
£7,350 to £8,722

Starting salary will be related to qualifications and experience. The salary scale is under review from 1 January 1980.

In addition there is a non-contributory pension scheme apart from a 1½% deduction from salary to cover widow's and dependant's benefits in the case of male officers.

The Commissioners may decide to interview only those applicants who appear from the information available, including level of academic attainments and experience, to be best qualified.

Further details of the work of the College and its Poultry Department are available on request from the Principal, Loughry College of Agriculture and Food Technology, Cookstown, County Tyrone BT80 9AA (telephone Cookstown 62491).

Please write or telephone for an application form quoting job reference SB 312/79/NT to the Civil Service Commission, Rosepark House, Upper Newtonards Road, Belfast BT4 3NR (telephone Dundonald 4585 ext 256). Completed forms must be returned to arrive not later than 12 October 1979.

3302(A)

NORTHERN IRELAND CIVIL SERVICE

STATE UNIVERSITY OF NEW YORK

DEPARTMENT OF CHEMISTRY

TENURE TRACK FACULTY POSITIONS

Assistant Professorships in Analytical and Inorganic Chemistry. Applicants for these positions must be committed to excellence in research and teaching. Areas of research should compliment those presently represented in the Department. A complete curriculum vita, which includes a documented summary of past accomplishments and professional references should be submitted to: Chairman, Search Committee, Department of Chemistry, State University of New York at Buffalo, Buffalo, New York 14214. Deadline for applications is January 15, 1980. The State University of New York at Buffalo is an equal opportunity/affirmative action employer. W386(A)

UNIVERSITY OF DURHAM

DEPARTMENT OF PHYSICS

Applications are invited for a

POSTDOCTORAL SENIOR RESEARCH ASSISTANT

tenable for three years and three months from 1 November 1979 to work with Professor B.H. Bransden in the Department of Physics on the Theory of Atomic Charge-Exchange Reaction, with special reference to Heavy Ion Fusion.

Initial salary in the range £4,333 to £7,227 on National Range 1A plus superannuation.

Applications (3 copies) naming three referees should be sent by 19 October 1979 to the Registrar and Secretary, Science Laboratories, South Road, Durham, DH1 3LE from whom further particulars may be obtained. 3276(A)

THE KENNEDY INSTITUTE OF RHEUMATOLOGY

A postgraduate

RESEARCH ASSISTANT

in the

DIVISION OF CELLULAR BIOLOGY

Applications are invited for the post of Research Assistant for six months, for research in cellular biology. The successful applicant will work under the Head of the Division, Dr J. Chayen. An interest in spectroscopy, optics and photography would be helpful.

Depending upon age and experience salary in the range £4,515 to £5,073 (inclusive of London Allowance), based on University of London scales.

Apply by letter enclosing curriculum vitae and the names of two referees, to The General Secretary, Kennedy Institute of Rheumatology, Bute Gardens, Hammersmith, W6 7DW. 3317(A)

Chemists for Drug Research

The Research Institute of Smith Kline & French Laboratories Limited, the UK based research group of the international SmithKline Corporation, is undertaking a planned expansion of its research activities as part of the move to a new 39 acre site in the grounds of a country house – The Frythe – in Welwyn. The new facility, being built to the highest standards of design, represents a major investment and will be totally dedicated to pharmaceutical research and development. Prior to the move to these new laboratories, we are recruiting chemists now as part of the first phase of expansion.

We are particularly interested in hearing from chemists with considerable experience of research in the pharmaceutical industry as well as newly qualified graduates and post-doctorals who wish to embark on a career in industry. This is an opportunity to join a successful basic research group with a broad interest in the biological effects of novel compounds related to specific hormone and transmitter action.

Currently we have the following vacancies:

Synthetic Organic Chemists

Vacancies exist in our Medicinal Chemistry and Chemical Support Departments for synthetic organic chemists, one of whom will be assigned to radiochemistry. Candidates should have a good background in organic chemistry and a flair for practical organic synthesis. Recently qualified candidates should preferably have an honours degree in Chemistry (MRIC, GRIC, B.Sc.) but, alternatively, we would also like to hear from candidates with HND/HNC coupled with relevant experience.

Medicinal Chemist

This post-doctoral vacancy presents an opportunity for a chemist to make a major contribution in synthesising novel biologically active molecules and, in collaboration with biologists and physical organic chemists, in relating biological activity to chemical properties. We invite applications from candidates with up to two years' post-doctoral experience who have demonstrable ability in organic synthesis. Research experience, preferably biologically-orientated and utilising physical-organic principles and techniques, would also be a distinct advantage.

Radiochemist

The radiochemical section has a vacancy for a graduate or Ph.D. to synthesise specifically radiolabelled molecules which may involve devising new routes towards them. Ability in small-scale organic synthesis is essential, and radiochemical training will be given if necessary.

Chromatographic Chemist

A vacancy now exists for a young graduate or equivalent with two to three years' relevant industrial experience to supervise an additional chromatography laboratory which is being set up in our Physical Organic Chemistry Department.

We offer competitive salaries on progressive incremental scales. Newly qualified Honours graduates will start on at least £4,500 and newly qualified Ph.D's at around £6,000. We operate flexible working hours and benefits include free life assurance amounting to three-times annual salary, an excellent contributory pension scheme and a discretionary annual bonus. We also give generous relocation grants to all appropriate candidates.

When replying to advertisement, please indicate which position is being applied for. Please write or telephone Mrs. P. E. Shaw, Personnel Officer – Research.

SMITH KLINE &FRENCH LABORATORIES LIMITED

Mundells, Welwyn Garden City, Herts. AL7 1EY. Tel: Welwyn Garden 25111. Ext: 26.

SK&F

a SmithKline company

1978 1979

3343(A)

CARDIOTHORACIC INSTITUTE

(Associated with the National Heart and Chest Hospitals)

TECHNICIAN

Qualified technician with relevant HN and a knowledge of immunological/bic chemical techniques, required to work o problems related to the "in vitro" assessment of the allergic patient in th Department of Clinical Immunology.

Salary on MLSO scale. Apply, quotin reference C12, to Secretary Cardiothoracic Institute, Fulham Roac London, SW3 6HP, stating age, experienc and qualifications and giving names of tw referees. 3318(A)

UNIVERSITY OF BIRMINGHAM

ASSISTANT SAFETY ADVISER

Applications are sought for the post Assistant Safety Adviser from scien graduates or professionally qualifie persons, preferably with experience health and safety work. The duties will concerned with the use, handling, stora and disposal of biologically hazardo materials. If necessary additional traini will be provided.

Salary in the scale £7,227 to £8,892 p. or for a less experienced candidate £3,7 to £7,521 p.a.

Further particulars and applicati forms available from The Senior Assista Secretary, University of Birmingham, P. Box 363, Birmingham B15 2TT, to who application should be made by 22 Octob 1979. 3301(A)

THE LONDON HOSPITAL MEDICAL COLLEGE

(University of London)

RESEARCH BIOCHEMIST PHYSIOLOGIST

Applications are invited for the above po Candidates should be a graduate in one, least, of the above subjects and should interested in gastrointestinal research. experience is necessary, but the pers appointed will be required to join in wo carried out by experimental groups cov ing biochemical and electrophysiologi experimental studies on the gastrointesti tract. Initial salary within the range £3, to £5,488 plus £740 London Allowance annum. Further details from Profess H.D. Ritchie, tel. 01-247:5454 Ext.407.

Applications, quoting Ref. RB/P/9/ together with names of two referees, to Secretary, London Hospital Medi College, Turner Street, London E1 2A within 14 days. 3305(A

UNIVERSITY OF BRISTOL

CHAIR IN VETERINARY MEDICINE

The University invites applications appointment to the Chair in Veterin Medicine which will become vacant on August 1980, following the retirement Professor C.S.G. Grunsell.

Candidates, who must have a veterin qualification, are invited to sub applications, including the names of th referees, not later than 30th Novem 1979.

Further particulars of the appointm may be obtained from the Registrar Secretary of the University, Senate Hou Bristol BS8 1TH. 3291(A

CHELSEA COLLEGE
(University of London)

CENTRE FOR SCIENCE EDUCATION

he Centre has the following vacancies:-
xperienced

STORES TECHNICIAN GRADE 4.

he responsibilities cover the operation, including unter service, of the Centre's general equipment d stationery stores with the minimum of direct pervision. The stores are self-financing and fer a wide range of materials and services.

The successful applicant must be adaptable, ethodical and able to communicate easily with dents, staff and visitors. Six years experience cluding a City and Guild or equivalent alification are required.

TECHNICIAN GRADE 3

cover laboratory work associated with st/undergraduate teacher-training and earch in Chemistry and chemically-related jects, e.g. Earth Sciences, Metallurgy, Food ence, etc.

Applicants should be adaptable and able to nmunicate easily with students and visitors, an perience of modern School teaching methods d equipment would be an advantage. Four years evant experience including O.N.C. or ivalent qualification are required.

TECHNICIAN GRADE 3

he Integrated Science Section. The successful plicant will work with the Senior Technician in Section which deals with a wide range of ence disciplines from Junior School to Sixth m level and the post will require a sound eral science background. In addition, the hnician will have a major responsibility for the ration of the Integrated Science Resources a. At least four years relevant experience, uding O.N.C., City and Guilds or equivalent required.

alary Scales: (under review)
Grade 4: £3,956 to £4,474 inclusive
Grade 3: £3,646 to £4,077 inclusive

urther details and application forms from the sonnel Officer, Chelsea College, Friese Greene ise, Chelsea Manor Street, London SW3 V. Tel: 01-352-1472 ext. 55. 3342(A)

UNIVERSITY COLLEGE LONDON

DEPARTMENT OF BIOPHYSICS

TECHNICIAN

Grade 3

ferably with experience in tissue culture histology to assist in nerve-muscle arch. Appointment for one year with sibility of extension. Salary £3,205 + 4 London Weighting.

pplications to Professor R. Miledi, t. of Biophysics, University College don, Gower St, London WC1E 6BT.
3266(A)

KING'S COLLEGE HOSPITAL MEDICAL SCHOOL
(University of London)
Denmark Hill, London,
SE5 8RX.

LIVER UNIT

lications are invited for the post of

POSTDOCTORAL RESEARCH FELLOW

join a multidisciplinary team stigating the aetiology of treatment of cancer. The successful applicant will a key role in the development of this y established group. The post will lve extensive use of cell culture niques and experience in this area is ntial.

lary within the range £5,488 to £7,521 ondon Weighting (under review).

pplications, giving full curriculum and the names and addresses of 2 ees should be sent to the Secretary of Medical School as soon as possible.
3307(A)

UNIVERSITY OF THE WEST INDIES — JAMAICA

Applications are invited for the post of

PROFESSOR/SENIOR LECTURER IN INORGANIC CHEMISTRY, CHEMISTRY DEPARTMENT.

The appointee will be expected to shape and teach undergraduate courses as well as to develop the graduate programme and will be expected to lead in the further development of research in the Department in the field of Inorganic Chemistry. Salary Scales:- Professor 1979/80 J$20,770 to 25714 pa, 1980/81 J$22,848 to 28,284 pa. Senior Lecturer: 1979/80 — J$15,489 to 19,530 pa, 1980/81 — J$17,748 to 21,717 pa (£1 sterling = J$3.00). Family passages; baggage allowance; FSSU.; study and travel grant; unfurnished accommodation will be let by the University at a rental of 10% of salary or a housing allowance of 20% of salary is payable.

Detailed applications (2 copies) with curriculum vitae and naming three referees should be sent as soon as possible to the Registrar, University of the West Indies, Mona, Kingston 7, Jamaica.

Applicants resident in the UK should also send one copy to the Inter-University Council, 90-91 Tottenham Court Road, London W1P 0DT.

Further details may be obtained from eiher address. 3323(A)

UNIVERSITY OF LEUVEN
Belgium
RESEARCH ASSOCIATE IN VIROLOGY

Applications are invited for the post of Research Associate, tenable for one and preferably two years with extensions possible. The programme is in the broad field of viral hepatitis, including nonA, nonB hepatitis, models of hepatitis-B related hepatoma, and antivirals. It can be expanded to related areas.

Research experience in virology is highly desirable, and proficiency in biochemistry, immunology or electron microscopy would be important assets. Normally only candidates who have passed a doctoral thesis will be considered, and those with postdoctoral experience will be preferred. A working knowledge of English is required.

Commencing salary is equivalent to 18,000-30,000 US dollars per annum, depending upon experience and qualifications. It is indexed to the cost of living, and social provisions including health insurance are as for Belgian nationals.

The position is open immediately. Applicants are invited to submit now their curriculum vitae, list of publications, addresses of two referees and the date from which they would be free to Professor J. Desmyter, Rega Institute for Medical Research, Minderbroedersstraat 10, B-3000 Leuven, Belgium. W387(A)

IMPERIAL CANCER RESEARCH FUND

PROTEIN CHEMISTRY LABORATORIES

SENIOR TECHNICIAN

required by our Protein Chemistry Laboratories to take responsibility for the operation and maintenance of equipment involved in determination of protein structure. Includes Beckman Sequencer, Durrum amino acid analyser and Waters high pressure liquid chromatography apparatus.

HNC/Degree, experience of complex instrumentation desirable. Capability to carry out basic electronic and mechanical maintenance essential. Opportunity to be involved in design and assembly of new equipment.

Salary range £5,370 to £6,939. For further information and application form write or telephone Miss S.M. Hurley, Imperial Cancer Research Fund, Lincoln's Inn Fields, W.C.2. on 242 0200 ext. 305, quoting reference 412/79. 3321(A)

Daresbury Laboratory is developing major national facilities for use by university groups carrying out research in atomic, molecular and biological sciences, nuclear physics and computational science. To provide for the computing needs of scientists working in these fields the Laboratory operates an extensive network of mini-computers, terminals and RJE stations centred on a large IBM 370/165 computer. The recent addition of a CRAY-1 computer has made the installation at Daresbury one of the most powerful in the country, and additional computer staff (male/female) are now sought to cope with the increasing work.

SYSTEM PROGRAMMERS

are required to join a small group engaged in extending and improving the operating systems on IBM, GEC and CRAY computers, where the areas to be strengthened include networking, timesharing, on-line data acquisition and compilers. Successful applicants can expect to work primarily in one or more of these fields, but with ample opportunity to gain experience in almost every aspect of computing. Staff are also encouraged to maintain an interest in the scientific work of the Laboratory.

Previous experience with operating systems is not essential, but a sound knowledge of computing concepts is desirable. The successful candidates will possess a good honours degree in a scientific discipline, a strong and growing general interest in both computers and science and a firm ambition to build a career in scientific computing.

Appointments will be made in the Scientific Officer grade in the salary range £3,289-£5,085 which will increase from 1st January 1980 as follows: £3,591-£5,486.

There is a non-contributory superannuation scheme, a generous leave allowance and a flexible working hours scheme.

Please write or telephone Warrington 65000 Ext. 467 for an application form quoting reference no DL/704/CB to:

The Personnel Officer
DARESBURY LABORATORY
Science Research Council
Daresbury, Warrington WA4 4AD

Closing Date: 26th October 1979.

3286(A)

UNIVERSITY OF EDINBURGH
LECTURESHIP IN PHYSIOLOGY
(£4,333 to £8,992)

Applicants must have a good Honours degree in Physiology and at least 3 years' research experience. Further particulars can be obtained from the Secretary to the University, Old College, South Bridge, Edinburgh EH8 9YL, to whom applications, including a full curriculum vitae and the names and addresses of 3 referees, should be sent not later than 20.10.79. Please quote Reference 1077. 3326(A)

POSTDOCTORAL POSITION — BIOCHEMIST. Available for 2 year appointment to study interrelationships between RNA and cancer. PhD in biochemistry or other related fields with strong background in biochemistry. Salary range $11,000 to $13,000 depending on experience. Submit curriculum vitae and supporting documentation to: Personnel Management Branch, College of Medicine & Dentistry of New Jersey — Rutgers Medical School, PO Box 101, Piscataway, NJ 08854. Equal Opportunity/Affirmative Action Employer M/F. W391(A)

DEPARTMENT OF AGRICULTURE FOR NORTHERN IRELAND

Senior Scientific Officer/ Higher Scientific Officer/ Scientific Officer
£3,289 to £7,864
(£3,591 to £8,705 from 1 January 1980)

Applications are invited for a pensionable post in the Field Botany Research Division of the Department of Agriculture, situated at the Plant Testing Station, Crossnacreevy, Castlereagh, Belfast.

DUTIES

Duties will involve the supervision of a small team carrying out trials to assess the merit of cultivars of agricultural crops especially grasses and clovers. The successful applicant would in due course be expected to initiate and carry out relevant applied research and to undertake advisory duties. The successful candidate may also be required to undertake lecturing duties in the Faculty of Agriculture and Food Science, Queen's University of Belfast.

Appointment may be at Senior Scientific Officer/Higher Scientific Officer/Scientific Officer level.

QUALIFICATIONS

Senior Scientific Officer

Over 25 years and under 32 years of age on 31 December 1979 with a first or second class honours degree in Agricultural Botany or a closely related discipline and at least 4 years' post graduate experience preferably concerned with the evaluation of crop cultivars. Knowledge of plant genetics and/or biometrics would be an advantage.

Higher Scientific Officer

Under 30 years of age on 31 December 1979 with an honours degree as above and at least 2 years' relevant post-graduate experience.

Scientific Officer

Under 27 years of age on 31 December 1979 with an honours degree as above.

Exceptionally applications may be considered from candidates over the age limits who have specialised experience.

The Civil Service Commissioners may decide to interview only those applicants who appear from the information available. (including level of academic attainment and relevant experience) to be best qualified.

SALARY SCALES

Senior Scientific Officer — £5,876 to £7,864. (£6,332 to £8,705 from 1 January 1980)
Higher Scientific Officer — £4,727 to £6,211. (£5,097 to £6,737 from 1 January 1980)
Scientific Officer — £3,289 to £5,085. (£3,591 to £5,486 from 1 January 1980)

There is a non-contributory pension scheme apart from a 1½% deduction from salary to cover widow's and dependants' benefits in the case of male officers.

Please write or telephone for an application form quoting job reference SB 337/79/NT to the Civil Service Commission, Rosepark House, Upper Newtonards Road, Belfast BT4 3NR (telephone Dundonald 4585 ext 256). Completed forms must be returned to arrive not later than 11 October 1979.

3303(A)

NORTHERN IRELAND CIVIL SERVICE

UNIVERSITY OF BERN
Switzerland
INSTITUT OF PLANT PHYSIOLOGY
POSTDOCTORAL RESEARCH ASSISTANT

Position available immediately for PhD with strong background in biochemistry an physiology of plant metabolism, to work on a project dealing with regulatin of carbon and nitrogen metabolism in green leaves.

The appointment will be for 1 year initially. Starting salary: Swiss Fr. 2'700.--to Fr. 3'000.-- per month (£745 to £838 per month).

Applicants are assisted in receiving permission to enter Switzerland and to attend to the necessary formalities with the foreign police.

Please send application with curriculum vitae, details of work experience and references to:
Prof. Dr K.H. Erismann, Intitut of Plant Physiology, University of Bern, Altenbergrain 21, CH - 3013 Bern, Switzerland. W381(A)

THE UNIVERSITY OF OXFORD
in Association with Brasenose College
UNIVERSITY LECTURERSHIP IN ANATOMY

The University proposes to appoint a lecturer in the Department of Human Anatomy to take up his/her post with effect from 1 April 1980 or as soon as possible thereafter. Salary according to age on the scale £4,333 to £9,581 (interim scale effective from 1 October 1979). The post may be held in association with a tutorial fellowship at Brasenose College.

Further enquiries concerning the lectureship should be addressed to Professor C.G. Phillips, F.R.S., Department of Human Anatomy, South Parks Road, Oxford, to whom applications (8 copies, one in the case of overseas candidates) together with the names of three referees should be sent by 31 October 1979.

Enquiries concerning the tutorial fellowship should be addressed to the Principal, Brasenose College, Oxford, by the same date. 3322(A)

UNIVERSITY OF LIVERPOOL
DEPARTMENT OF MICROBIOLOGY

Applications are invited for the post of

RESEARCH ASSISTANT/ SENIOR RESEARCH ASSISTANT

in the Department of Microbiolog Candidates should hold or be about receive, a PhD degree. The project funded by the M.R.C. for a period of thr years. The research concerns the uptal and establishment of plasmid DNA durir transformation of *Escherichia coli* ar other Gram negative bacteria, collaboration with Dr G.O. Humphre and Dr J.R. Saunders.

The initial salary will be £3,775, £4,0 or £4,333 per annum plus USS benefit The appointment is from 1st Octobe 1979, or as soon as possible thereafter.

Applications, together with the names three referees, should be received as soon possible by The Registrar, The Universit P.O. Box 147, Liverpool, L69 3BX, fro whom further particulars may be obtaine Quote Ref. RV/791/N. 3304(A)

JUSTUS-LIEBIG-UNIVERSITY OF GIESSEN, FRG
INSTITUTE OF VIROLOGY
POST-DOCTORAL VIROLOGIST OR IMMUNOLOGIST

required to join a group engaged in t study of the immunological and biologic aspects of tumor cell surface antigens Rous sarcoma virus transformed cel Aspects of study include 1) antige analysis and production of monoclo antibodies against antigens coded for t virus and cell, respectively. 2) Significan of the antigens for the malignant state the cell. 3) Transplantation immunizati experiments in vivo.

Applicants should possess experien either in virology, immunology biochemistry. The appointment is for thr years with the possibility of extension another two years period.

Applications, with curriculum vitae, l of publications and the name of tv referees should be made within four wee to Prof. Dr Heinz Bauer, Institut f Virologie, Fachbereich Humanmediz Frankfurter Str. 107, 63 Giessen, FRG. W380(A)

HEAD OF PHARMACOLOGY DEPARTMENT

A leading Spanish Pharmaceutical Company situated in the mediterranean city of Barcelona invites applications from pharmacologists for the above post in a Pharmacology Department. The man we appoint will have a good honours degree in an appropriate discipline, a PhD in pharmacology and several years post-doctoral experience preferably in the pharmaceutical industry.

The successful applicant will be responsible to the director of Biological Sciences and must be able to formulate and organise research and development projects and collaborate with the other departments in the Biological and Chemical science groups.

Knowledge of Spanish is not initially essential but the successful candidate must be prepared to acquire adequate fluency in this language within 2 years. The salary is of US$40,000 pa, tax free and special assistance will be given for removal expenses to Barcelona.

Applications in writing with full curriculum vitae should be addressed to:

Pharmacology Department
P.O.B. 1583
BARCELONA-24 (Spain) W398(A)

UNIVERSITY OF OXFORD

In association with
St. Hilda's College or
New College

UNIVERSITY LECTURERSHIP in ORGANIC CHEMISTRY

pplications are invited for the above post be filled from 1 October 1980. Stipend cording to age on the scale £4,232 to ,000. The successful candidate may be fered a tutorial fellowship by St. Hilda's llege (for a woman) or New College (for nan). Details of the university and college pointments may be obtained from the lministrator, Dyson Perrins Laboratory, uth Parks Road, Oxford OX1 3QY, to om completed applications (six typed pies, only one from overseas candidates) ould be sent by December 31, 1979.

3282(A)

UNIVERSITY OF SOUTHAMPTON

ANCER RESEARCH CAMPAIGN
MEDICAL ONCOLOGY UNIT

plications are invited for the position of st doctoral research fellow to work on chanisms of resistance to anti-cancer gs. Candidates should have, or be about obtain, a PhD in Biochemistry or armacology. Some experience of tissue ture would be an advantage. The pointment will be for 3 years and the cessful applicant will be expected to e up his or her duties as soon as veniently possible.

Salary: £4,333 to £5,777 p.a. on Range , USS benefits.

Applications giving date of birth, riculum vitae and the names of three erees should be sent to Mrs P. Vaughan-ith, Staffing Section, The University, ithampton, SO9 5NH, as soon as sible quoting ref: 1099/R/Na

3328(A)

SUNDERLAND POLYTECHNIC

Faculty of Science
DEPARTMENT OF BIOLOGY

SENIOR TECHNICIAN (SR)

ary scale: T3/4 £4,080 to £5,067 per um.

his postholder carries a special onsibility for electron microscopy. erience in preparation of biological erials and routine maintenance of smission microscopes is required.

n application form may be obtained n the Personnel Officer, Sunderland ytechnic, Chester Road, Sunderland, 3SD, and should be returned as soon as ible.

3337(A)

UNIVERSITY OF BRISTOL

CHAIR IN VETERINARY SURGERY

University invites applications for ointment to the Chair in Veterinary gery which will become vacant on 1st uary 1980, following the retirement of fessor G. Arthur.

didates, who must have a veterinary lification, are invited to submit ications, including the names of three rees, not later than 30th November .

her particulars of the appointment be obtained from the Registrar and etary of the Univesity, Senate House, tol BS8 1TH.

3290(A)

INSTITUTE FOR BIOTECHNOLOGICAL RESEARCH LTD

(Gesellschaft für Biotechnologische Forschung mbH,
Braunschweig, Germany)

A position is immediately available in the above Institute for a

POSTDOCTORAL RESEARCH ASSISTANT

for work on diagnosis of leucoses by cell surface markers, and monoclonal antibodies against serum components. Applicants should hold a degree in Biochemistry or Medicine.

Salary will be from 40.880, -- DM to 48,250, -- DM per annum. Applications with curriculum vitae and publication list should be sent not later than December 1st. 1979 to the Gesellschaft für Biotechnologische Forschung mbH, Mascheroder Weg 1, D 3300 Braunschweig-Stöckheim, Germany.

W397(A)

IMPERIAL COLLEGE GRADE 5 TECHNICIAN

Required to work with Dr K.W. Buck and Dr R.H.A. Coutts on cenome organisation and expression of a DNA plant virus. Experience in nucleic acid, protein or virus biochemistry would be an advantage. Salary in the range £3,700 to £4,320 (under review 1.10.79 with further minimum increase of £226 to £264 from 1.4.80). Plus London weighting of £524 (under review). Superannuation, sick pay scheme, 37 and a half hour week. 5 weeks annual leave plus additional days at Christmas and Easter. Modern staff club and excellent facilities, with sports centre, swimming pool.

Application forms can be obtained from: Departmental Superintendent, Biochemistry Department, Imperial College, London SW7. Tel: 01-589 5111 ext. 1119.

3341(A)

SCIENTISTS FOR SAFETY TECHNOLOGY RESEARCH AND DEVELOPMENT

PHYSICISTS AND MATHEMATICIANS

are needed by the

SAFETY AND RELIABILITY DIRECTORATE

of the UKAEA at Culcheth, Warrington,

to work in multi-disciplinary teams on varied technical studies of, and research on the safety and reliability of all types of nuclear reactor systems, and other large scale plants (not necessarily nuclear). Principal objectives are the continual updating of safety and reliability philosophy by anticipating important problems which may arise from technological advances; followed by solutions that can be applied to engineering design and environmental planning. In following these aims there are extensive programmes of collaboration with many overseas countries, and other organisations within the United Kingdom.

Candidates are normally expected to hold honours degrees in relevant subjects, and should have clear interests in advancing safety technology.

Appointments, as SCIENTIFIC OFFICERS, are open to persons of either sex. The current salary range is £3,520 to £5,445 pa, but this is due to be revised to £3,845 to £5,875 from 1.1.80. On the current range a candidate with a first or upper second class honours degree and a year's relevant post-graduate experience could expect a starting salary of £4,285 pa. (£4,870 from 1.1.80.)

Some higher grade posts may be available for people with a few years suitable post-graduate experience.

APPLICATION FORMS and further information obtainable from: Adrian Wilson, The Personnel Officer, UKAEA, SRD, Wigshaw Lane, Culcheth, Warrington WA3 4NE, quoting reference B38/J14.

3287(A)

Wellcome

Biochemist £6000-£7000

The Wellcome Foundation Limited is a major British-owned international pharmaceutical organisation producing a wide range of chemical and pharmaceutical products.

We currently have an opportunity for a biochemist with extensive experience in enzymology to classify the mechanism of action of specified drugs by defining their interactions with enzymes. The successful candidate will work in close liaison with chemists and biochemists and is expected to have a considerable input in the overall design and direction of the work.

Candidates ideally will have skills equivalent to that of a 4 year post-doctoral biochemist/enzymologist and a publication list demonstrating originality in research. The post is at present in the Department of Biochemical Research at Dartford in Kent, which will be relocated in Beckenham in the near future.

We offer a salary in the range £6000 to £7000 depending on age, qualifications and experience. Excellent benefits include 4 weeks' holiday, life assurance and sick pay schemes, excellent sports and social facilities and generous assistance with relocation expenses, where appropriate.

For an application form, please write to or ring
Ian Griffiths, Personnel Officer,
The Wellcome Foundation Limited,
Temple Hill, Dartford, Kent.
Tel: Dartford (32) 23488, ext. 2061.

3333(A)

VIROLOGIST
Assistant Professor

Two-year appointment with the possibility of tenure track. Salary commensurate with experience. To teach 2 half year lecture courses in Virology at the third and fourth year levels plus 1 half year Virology techniques laboratory. The candidate should have a PhD, some post-doctoral experience and be capable of developing a research program in Virology.

Excellent research facilities including Tissue Culture, Radioisotope and Biohazard containment laboratories are available.

The position is to start January 1, 1980, or as soon as a suitable candidate can be found.

Curriculum Vitae with names of 3 referees should be sent not later than December 15, 1979 to:

Chairman,
Department of Biology,
University of New Brunswick,
Fredericton, N.B.,
Canada E3B 5A3

W371(A)

SCOTTISH MARINE BIOLOGICAL ASSOCIATION
DUNSTAFFNAGE MARINE RESEARCH LABORATORY
nr. Oban, Argyll.

Applications are invited for the post of

DEPUTY DIRECTOR

at the Dunstaffnage Marine Research Laboratory.

The Dunstaffnage Laboratory is multidisciplinary, undertaking basic, strategic and applied research in coastal waters and the deep sea. Some postgraduate teaching is undertaken in conjunction with Stirling University. The staff number about 100 and in addition there are about 12 postgraduate students. The Laboratory, which is independent, is funded by a grant from the Natural Environment Research Council and by contract work, and has an annual budget of about £950,000.

Candidates will be expected to have an established reputation in marine science and an aptitude for laboratory administration. The post is graded at Senior Principal Scientific Officer level, and the appointee will be required to take part in the management of the research and administration of the Laboratory and deputise for the Director in his absence.

Application forms and further details may be obtained from the Administrative Officer, Dunstaffnage Marine Research Laboratory, P.O. Box 3, Oban, Argyll PA34 4AD. Closing date for receipt of application forms: 26 October, 1979.

3314(A)

OVERSEAS DEVELOPMENT
KNOW-HOW-vital to developing countries

Oilseeds Agronomist — Tanzania

As member of Oilseeds Research Team carrying out breeding and agronomy projects in Southern Tanzania. Duties:— carry out agronomic investigations on sesame, sunflower and groundnuts in Mtwara, Lindi and Rovuma Regions, assist with regional trials on these crops in substations and villages with particular emphasis on role of the three crops in farming practices and systems of area, train Tanzanian staff in these disciplines and assist in general development of agronomic aspects of Project. Candidates, should possess Honours degrees in Agriculture or Natural Sciences. Appropriate postgraduate qualification and previous overseas experience desirable.

Appointment: 2 tours of 1 year. Salary (UK taxable) in range above £8,000 pa plus a variable tax free overseas allowance in range £1,780 — £4,330 pa (Ref 331X).

The post is wholly financed by the British Government under Britain's programme of aid to the developing countries. In addition to basic salary and overseas allowances other benefits normally include paid leave, free family passages, childrens education allowances and holiday visits, free accommodation and medical attention. Applicants should be citizens of the United Kingdom.

For full details and application form please apply, stating post concerned, and giving details of age, qualifications and experience to:—

Appointments Officer,
OVERSEAS DEVELOPMENT ADMINISTRATION,
Room 301, Eland House,
Stag Place, London SW1E 5DH.

3332(A)

ODA

HELPING NATIONS HELP THEMSELVES

WELSH NATIONAL SCHOOL OF MEDICINE
(University of Wales)
DEPARTMENT OF MEDICAL BIOCHEMISTRY

Applications are invited for the post of

RESEARCH OFFICER

in the above Department at Heath Par
Cardiff to work on the regulation of intr
cellular calcium in exocrine cells and its rc
in the stimulation of secretion. The proje
is funded by the M.R.C. for a period
three years and might be suitable for a bi
chemistry graduate wishing to
considered as a candidate for a high
degree.

Salary will be £3,775 per annum on the sc
for University Research and Analogo
Staff Grade 1B.

Further particulars and application for
may be obtained from the Registrar a
Secretary, Welsh National School
Medicine, Heath Park, Cardiff quoti
ref. No. C38/4/27. 3289(A)

THE CAMPDEN FOOD PRESERVATION RESEARC ASSOCIATION
Chipping Campden
Glos., GL55 6LD
(Telephone Evesham (0386) 840319

requires a Physical Scientist f
development in pilot plant area
preservation technology. Some work
factory environment can be anticipated.

Candidates should be graduates with
interest in food preservation and prov
ability to formulate project detail a
analyse results. Training will be giv
Appointments on Research Officer sc
£3,048 to £5,316 pa. Application form a
job specification on request to Secreta
General. 3339(A)

MEDICAL RESEARCH COUNCIL
LABORATORY OF MOLECULAR BIOLOGY

Applications are invited for a scienti
appointment:

Post available from 1 October 1980,
up to five years in the first instan
Candidates must have at least an up
second class honours degree and seve
years relevant postgraduate experience.

The person appointed will work on
structural aspects of cell motility, w
special emphasis on electron microscop

Salary based on University lectures
rates.

Applications, with full c.v. and names
two referees, should be sent to
Administrative Secretary, M
Laboratory of Molecular Biology,
Medical School, Hills Road, Cambri
CB2 2QH, to be received not later than
October 1979. 3325(A

CHARING CROSS HOSPITAL MEDICAL SCHOOL
(University of London)
RESEARCH TECHNICIA

Applications are invited from suita
qualified people for the post of Resea
Technician to be appointed for one yea
the first instance primarily to help in
investigation of lipid abnormalities
patients with cerebrovascular disease, a
some enzymology involved. Holder of p
will be expected to develop own lines
research within this field.

Salary on scale £3,768 to £5,391
£354 London Weighting Allowance
annum.

Applications on forms quoting
039/40, obtainable from The Secreta
Charing Cross Hospital Medical Scho
The Reynolds Building, St. Dunsta
Road, London, W6 8RP., to be retur
within three weeks of the appearance of
advertisement. 3335(A

AGRICULTURAL RESEARCH COUNCIL

FOOD RESEARCH INSTITUTE

CHEMISTRY AND BIOCHEMISTRY DIVISION

(Physical Methods Groups)

SCIENTIFIC OFFICERS

1. A Biophysicist or Physical Chemist is required to join a team to initiate a programme of research into the molecular basis of the functional behaviour of biopolymers in food systems. Ref: 79/12

2. A Physical Chemist is required to join a newly-constituted team to study the physiochemical properties of dispersions and gels in food.. Ref: 79/10

Qualifications:

First or Upper Second Class Honours degree in Physics, Chemistry or Biochemistry. Previous experience in surface chemistry or in the use of physical methods n Biology will be an advantage.

Salary:

From 1st August, 1979: on a scale £3,289 to 5,085.

From 1st January 1980: on a scale £3,591 to 5,486.

Five day working week and flexible working hours scheme operated. Transport facility available mornings and evenings.

Further particulars and application form from the Secretary, Food Research nstitute, Colney Lane, Norwich NR4 7UA quoting appropriate reference. Closing ate: 11th October 1979. 3299(A)

THE UNIVERSITY OF MANCHESTER

LECTURER IN BIOCHEMISTRY

pplications invited from post-doctoral candidates with research experience in any branch of biochemistry for this post in the Department of Obstetrics and Gynaecology, at St. Mary's Hospital. Salary range p.a.: £4,333 to £8,992. Superannuation. Particulars and pplication forms (returnable by December st) from the Reigstrar, The University, Manchester, M13 9PL. Quote ref 212/79.

Requests for specific details about boratory facilities and research activities ould be addressed to Dr W.G. Bardsley, epartment of Obstetrics and ynaecology, St. Mary's Hospital, anchester, M13 0JH. 3296(A)

TOPEXPRESS LTD

Scientific and Computer Consultants seeks

BRIGHT AND ENTERPRISING PEOPLE

to work in the areas of vibration and acoustics. Research experience (PhD or equivalent), theoretical or experimental, would be a decided advantage. Salary negotiable, but generous. Pleasant informal working conditions in the centre of Cambridge.

Applications with curriculum vitae and names of two referees to Dr J. Woodhouse, Topexpress Ltd, 1 Portugal Place, Cambridge CB5 8AF.

3309(A)

UNIVERSITY OF BRISTOL

Applications are invited from recent graduates for the post of

DEMONSTRATOR in the DEPARTMENT OF GEOGRAPHY.

The post is tenable from the 1st October 1979, and will be for one year in the first instance, but renewable annually up to a maximum of three years.

Candidates should hold a good honours degree in Geography (or a related field) with special interests in Physical Geography, and time will be made available for research for a Higher Degree.

Commencing salary within the range £3,775 to £4,055 to £4,333 p.a. according to age and experience.

Applications, together with the names of two referees, should be sent to the Registrar and Secretary, University of Bristol, Senate House, Bristol BS8 1TH, as soon as possible (please quote reference EB). Any enquiries regarding this appointment should be addressed to the Head of Department of Geography. 3292(A)

UNIVERSITY OF BIRMINGHAM

DEPARTMENT OF CARDIOVASCULAR MEDICINE

Applications are invited for the post of

RESEARCH STUDENT

from persons with a good honours degree in Biochemistry, Physiology or related subjects to study aspects of the contractile and regulatory proteins of the human myocardium in normal and pathological conditions. The post which is supported by the British Heart Foundation is available from October 1979 and is funded for 3 years.

Applicants will be expected to register for the degree of PhD.

Further details can be obtained from Dr Peter Cummins, Department of Cardiovascular Medicine, Clinical Research Block, Queen Elizabeth Hospital, University of Birmingham, Birmingham B15 2TH, to whom applications, together with curriculum vitae and names of two referees should be returned not later than Friday 19th Octoer. 3313(A)

THE QUEEN'S UNIVERSITY OF BELFAST

Research Assistant — Radiocarbon Dating Palaeoecology Laboratory

Required to assist with research into short term variation in atmospheric ^{14}C concentration and radiocarbon time-scale calibration. The work involves high precision ^{14}C measurement techniques using liquid scintillation analysis, gas proportional counting and mass spectrometry. A good honours degree in Physics, Chemistry or related disciplines is to be preferred.

Salary range: £3,775 to £4,333, initial placing depending on age and experience. The post is available for three years.

Applications, including curriculum vitae and the names and addresses of two referees should be addressed to the Personnel Officer, The Queen's University of Belfast. Closing date: 12th October, 1979.

3283(A)

LABORATORY SCIENTIFIC OFFICER required for two years for service work in connection with prenatal diagnosis of human neural tube defects. Apply in writing to the Administrative Assistant, Paediatric Research Unit, The Prince Philip Research Laboratories, Guy's Hospital Tower, London Bridge, SE1 9RT. 3298(A)

POST DOCTORAL POSITION/ RESEARCH ASSOCIATE. Available immediately. Research will involve molecular aspects of influenza virus, replication and studies on nucleic acids of influenza viruses. Contact: Dr. Peter Palese, Department of Microbiology, The Mount Sinai School of Medicine, One Gustave L. Levy Place, New York, NY 10029. An equal opportunity employer. W393(A)

ROYAL NAVAL COLLEGE, GREENWICH, LONDON SE10 9NN

Research Appointments in the Nuclear Marine Technology Unit (Department of Nuclear Science and Technology)

Applications are invited for the following posts in the Unit:

HIGHER RESEARCH SCIENTISTS

POST A – To study the transport and chemistry of radionuclides in the marine environment and to develop models of their dispersion.

POST B – To study the computer simulation of Pressurised Water Reactors dynamic behaviour including the sensitivity and validation of computer models, and to define the systems engineering requirements and supporting experimental programs.

RESEARCH SCIENTISTS

POST C – To study and develop the monitoring of neutron damage fluence in pressurised water reactor materials.

POST D – To study thin-film thermouminescent dosimetry and its application to interface dosimetry.

Appointment will normally be for a fixed period of up to 3 years.

QUALIFICATIONS & EXPERIENCE

Candidates must have First or Upper Second Class Honours degree in a relevant scientific subject (those shortly to qualify may apply). Candidates for Higher Research Scientist posts must also have at least 2 years post graduate experience preferably in the application of simulation or computer modelling in oceanography, in respect of Post A; and simulation and/or modelling in nuclear reactor technology, in respect of Post B.

SALARY

HIGHER RESEARCH SCIENTIST – From £4727-£6211 (to become £5097-£6737 from 1.1.80) } Plus Outer London Weighting

RESEARCH SCIENTIST – From £3289-£5085 (to become £3591-£5486 from 1.1.80) } allowance of £325 per annum

Starting salaries according to qualifications and experience.

The Department has excellent and comprehensive facilities for studies in nuclear technology and the marine environment, with new digital and hybrid computers; a research reactor and analytical laboratories, and has the ability to provide for experiments in the relevant environment to be carried out. There will be opportunities, where appropriate, for successful candidates to register for M.Phil. or Ph.D.(CNAA).

Single accommodation may be available in the College, which provides excellent sports and social facilities.

Request for further details and application forms should be made to:–

The Ministry of Defence, CM(S) 1b3,
Room 310 Savoy Hill House,
Savoy Hill, London WC2R 0BX. Reference 453/01/9

Completed application forms should be returned by 22nd October, 1979.

3308(A)

UNIVERSITY OF FREIBURG/BR

Applications are invited for the post of

POSTDOCTORAL RESEARCH FELLOW IN NEUROCHEMISTRY

to study mechanisms of peptidergic neurotransmission in mammalian brains. Curriculum vitae and references should be sent to:

Dr H. Cramer, Professor, Section on Neurochemistry, Department of Neurology, Hansastr. 9, D-7800 Freiburg, West Germany. W388(A)

Laboratory Scientific Officer required as soon as possible until 31st January 1981 for work in the field of histology and spectrophotometric analysis, in the Dental Clinical Research Laboratory. Salary for qualified Laboratory Scientific Officer not less than £3,788 (revising to £4,125 on 1st January 1980) plus £354 London Weighting. Apply in writing, stating age and giving details of qualifications and experience, to the Secretary, Guy's Hospital Medical School, London Bridge, SE1 9RT, quoting Ref. D.C.R. 3311(A)

UNIVERSITY COLLEGE CORK

BIOCHEMISTRY

Applications are invited for two research positions supported by grants from the National Board for Science and Technology. Applicants should have an honours degree in Biochemistry or Chemistry and preferably a higher degree.

The appointments are for one year in the first instance with the probability of extension for one or two further years. Salary will be in accordance with qualifications and experience.

Applications, including a curriculum vitae and the names of two referees should be sent to Professor Shawn Doonan, Department of Biochemistry, University College, Cork, Lee Maltings, Prospect Row, Cork. (Ireland). **3334(A)**

THE UNIVERSITY OF LEEDS

DEPARTMENT OF BIOCHEMISTRY

Applications are invited for a post of

RESEARCH FELLOW

in the Department of Biochemistry. The project, under the supervision of Dr S.B. Brown and supported by the Nuffield Foundation, will involve studies on the mechanisms of oxidative degradation of haem and chlorophyll derivatives. The post will be suitable either for an organic chemist interested in acquiring experience in a biochemical environment or, alternatively, for a biochemist with some competence in organic chemical techniques. Previous experience with tetrapyrroles would be an advantage but is not essential. A PhD degree is required. The appointment will be made for a fixed period of three years and it is hoped that the successful candidate will be able to take up the post as soon as possible.

Starting salary in the range £4,333 to £5,488 on the 1A scale for Research and Analogous Staff (£4,333 to £7,521) (under review) according to age, qualifications and experience.

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference number 83/25. Closing date for applications 19 October 1979.

3319(A)

The European Space Agency has the following vacancies in the Space Science Department at its Technological Centre in Noordwijk, The Netherlands:

scientists european x-ray observatory satellite exosat operations

Applications are invited from young scientists holding post-doctoral or equivalent qualifications in astronomy or physics and having experience with scientific instrumentation and data analysis (preferably related to space research) to act as duty scientists in the operation of the European X-Ray Observatory Satellite EXOSAT.

Duties include evaluation of the feasibility of observation proposals, establishment of observation programmes, liaison with observers, conduct of observations and preliminary data analysis. The scientists will have the opportunity of undertaking their own observation programmes in X-ray astronomy.

Prior to launch the scientists will be located at ESA's Space Science Department ESTEC, transferring to ESOC, Darmstadt for orbital operations.

Application forms and further details may be obtained from the Head of Personnel ESA/ESTEC, Noordwijk, The Netherlands.

W395(A)

IMPERIAL CANCER RESEARCH FUND

MEDICAL ONCOLOGY UNIT

at St. Bartholomew's Hospital

TECHNICIAN/ RESEARCH OFFICER

required by our Medical Oncolog Unit at St. Bartholomew's Hospita to continue the investigations of non Hodgkin Lymphoma by surfac marking techniques.

HNC/Degree with experience o immunology (immunofluorescenc rosetting techniques, complemen technology or tissue typing) essentia Salary range £4,122 to £5,949.

For further information and appli cation form write or telephone Mis S.M. Hurley, Imperial Cance Research Fund, Lincoln's Inn Field London, W.C.2. on 242 0200 ex 305, quoting reference 410/79.

3320(A)

THE UNIVERSITY OF MANCHESTER

BARKER CHAIR OF CRYPTOGAMIC BOTANY

Applications are invited for the Bark Chair of Cryptogamic Botany which w fall vacant on October 1st, 1980 followi the retirement of Professor John Colhou The University has a preference f candidates with interests in the appli fields. The other Chair in Botany is t George Harrison Chair to whic Dr Elizabeth G Cutter has been elect Particulars of the appointment may obtained from the Registrar.

Applications (2 copies, 1 suitable f photocopying), giving full details qualifications, experience, research et and the names and addresses of thr persons to whom reference may be mad should be sent to The Registrar, T University, Manchester, M13 9PL October 10th. 3159(A)

UNIVERSITY OF DUBLIN
Trinity College

and

GEOLOGICAL SURVEY OF IRELAND

MICROPALAEONTOLOGIST

A micropalaeontologist or palynologis with a minimum of three year postgraduate experience is required t work on contract for the Geologica Survey of Ireland. The successfu candidate will head a joint Geologica Survey/Trinity College Dublin tea making an integrated stratigraphica study of offshore Mesozoic basin based upon material obtained fro petroleum exploration wells.

The post, which will becom available in January, 1980, carries commencing salary of £7,000 p.a.

Further particulars are obtainab from

Professor C.H. Holland,
Department of Geology,
Museum Building, Trinity Colleg
Dublin 2,

to whom applications, including th names of three referees, should be se *not later than 30th October, 1979.*

3329(A)

ICI CORPORATE LABORATORY, RUNCORN

MOLECULAR BIOLOGIST

Applications are invited from molecular biologists with a minimum of two years' post-doctoral experience to join a research team interested in the control of expression of eukaryotic genes. Experience in the isolation of MRNA from mammalian tissue and techniques of DNA cloning are highly desirable.

The appointment will be on a permanent basis and the work will be situated in our laboratories at Runcorn where a GMAG approved category III laboratory is available. In addition, there will be opportunity to collaborate with the group working in the ICI Joint Laboratory in the Biochemistry Department at the University of Leicester.

Excellent career prospects exist and, in addition to a competitive salary, the Company has a profit sharing scheme and an excellent contributory pension scheme. Where appropriate, assistance will be given with relocation expenses.

Applicants should write, enclosing full curriculum vitae, to:—

Miss S E Bird
Personnel Officer
ICI Corporate Laboratory
P O Box 11 The Heath, RUNCORN
Cheshire

3293(A)

SEMINARS and SYMPOSIA

CHROMATOGRAPHY DISCUSSION GROUP
(LC sub-group)

One-day Symposium at Hatfield Lodge Hotel, Hertfordshire on November 29th, 1979.

BIOMEDICAL APPLICATIONS OF HPLC

Lecture programme and speakers:—

Drug and metabolite analysis in biological samples
  Mr G Merry (Waters)
Measurement of vitamin D metabolites in serum
  Mr T Clemens (Middlesex Hospital)
Drug metabolism studies using HPLC
  Dr T R Marten (Roche Products Ltd)
Determination of Ranitidine and its metabolites in body fluids
  Mr P Carey (Glaxo Group Res.)
Nucleoside and nucleotide analysis using reversed phase HPLC
  Dr R Hartwick (University of Edinburgh)
Theory of Isotachophoresis and its application to Biomedicine
  Mr J Heslop (LKB)
An automated system for pre chromatographic clean up of biological samples
  Dr G B Cox (Du Pont)

Symposium fee £12.50, non-members £17.50.

Application forms from Mrs J A Challis (Exec. Sec. CDG)
Trent Polytechnic, Burton Street,
Nottingham NG1 4BU
Tel. 0602-48248 Ext 2187

3294(M)

UCLA JERRY LEWIS NEUROMUSCULAR RESEARCH CENTER'S
Inaugural Symposium on Excitation-contraction coupling

will be held December 4-7, 1979, at UCLA. Topics for discussion: Electrophysiological Analysis of EC Coupling; Mechanisms at the Level of the T-SR Junction; Molecular Aspects of the Contractile Response.

For program and registration information, write: Alan D. Grinnell, PhD, Director Jerry Lewis Neuromuscular Research Center, UCLA School of Medicine, Los Angeles, California 90024.

W392(M)

FELLOWSHIPS

UNIVERSITY OF SOUTHAMPTON

SCHOOL OF BIOCHEMICAL and PHYSIOLOGICAL SCIENCES
BIOCHEMISTRY DEPARTMENT

POSTDOCTORAL RESEARCH FELLOW — BIOCHEMISTRY

Applications are invited for a Postdoctoral Fellowship in Biochemistry. This appointment is a MRC funded position, available for a postdoctoral biochemist to work in collaboration with Dr D P Bloxham on the development of techniques for the measurement of rates of hepatic glucose and glycogen metabolism. The position will be well suited to candidates with a research background in enzymology who wish to expand their interest in metabolic control. The appointment is for one year in the first instance, commencing as soon as possible, with the possibility of an extension for a further two years.

Starting salary £4,333 pa (under review wef 1 Oct 79) on Range 1A. USS benefits.

Applications giving date of birth, curriculum vitae and the names of three referees should be sent to Mrs P Vaughan-Smith, The University, Southampton, SO9 5NH by 15th October 1979. Please quote reference 1098/R/N. 3281(E)

-Unique Opportunity-

STANFORD UNIVERSITY
NSF POSTDOCTORAL FELLOWSHIP in MOLECULAR BIOLOGY and COMPUTER SCIENCE
$14,000 per annum

Applicants should be familiar with the techniques of modern molecular biology and be interested in entering the field of computer science. Previous programming experience is useful but not essential. Responsibilities will include development and maintenance of a knowledge base of current laboratory techniques for use with an advanced model of automated experimental planning. One goal of this program is to train an individual for a career in computer science as applied to molecular biology and genetics. While the fellowship will be awarded through the Department of Computer Science, the fellow will interact regularly with research groups in the Departments of Biochemistry and Medicine.

Send resume and the names of three references to either:
Dr Douglas Brutlag, Department of Biochemistry or Dr L.H. Kedes, Department of Medicine, Stanford University, School of Medicine, Stanford, California 94305. Equal opportunity through affirmative action. W383(E)

IMPERIAL CANCER RESEARCH FUND

RESEARCH FELLOWSHIP

A post doctoral fellow is sought to join a group working on the structure and function of paporavirus tumour antigens, with particular emphasis on the possible role of membrane associated protein kinases in virus induced transformation. Experience in cell biology, membrane biochemistry, protein kinases or enzyme purification might be advantageous.

Three year appointment. Salary range £5,800 to £7,100. Further information from Dr Alan E. Smith (01 242 0200 ext. 239). Applications with curriculum vitae and the names of two referees should be sent to the Personnel Officer, Imperial Cancer Research Fund, Lincoln's Inn Fields, W.C.2. quoting reference 394/79.

3225(E)

PORTSMOUTH POLYTECHNIC

BIOPHYSICS LABORATORIES

POST-DOCTORAL FELLOWSHIP in CHROMATIN RESEARCH

An SRC supported position is available until September 1981. The subject of study is the post-synthetic acetylation of histones, a chemical modification important in the control of genetic activity. The project is part of a large team effort devoted to the structure and function of chromatin. Candidates need not have experience in chromatin research but would be expected to have completed doctoral studies on a biochemical topic.

Salary: £4,722 to £4,845 per annum, plus an interim supplement at the rate of £72 per annum.

Application forms and further particulars from the Staff Officer, Portsmouth Polytechnic, Alexandra House, Museum Road, Portsmouth, PO1 2QQ, to whom completed applications should be returned by 11th October 1979.

Please quote reference no: S96.

3297(E)

RESEARCH FELLOWSHIP

A post-doctoral cytogeneticist or molecular biologist, with experience in human karyotyping, is required to participate in a project on the analysis of chromosomes from human solid tumours.

The appointment is for three years. Salary with entry according to qualifications and experience within range £5,990 to £7,434 inc. L.A. For further information telephone Dr E. Solomon 242 0200 extension 426.

Applications with curriculum vitae and names of two referees should be sent to the Secretary, Imperial Cancer Research Fund, Lincoln's Inn Fields, London, W.C.2., quoting reference 407/79. 3273(E)

NEW HALL, CAMBRIDGE
RESEARCH FELLOWSHIP

Applications are invited from graduates for a Research Fellowship in Science, tenable at New Hall for three years from 1st October 1980.

Application forms and further particulars may be obtained from the President, New Hall, Cambridge, CB3 0DF, to whom applications should be sent by 1st November 1979. 3338(E)

FELLOWSHIPS continued

UNIVERSITY OF BIRMINGHAM

DEPARTMENT OF BIOCHEMISTRY

RESEARCH FELLOWSHIP

A Research Fellow is required immediately for the period to 31 October 1980 for work supported by the Medical Research Council on hepatic glucokinase. The Fellow will work with Professor D G Walker and continue a project concerned with several aspects of the metabolic regulation of the enzyme.

Salary on scale starting at £4,333 (October scales) according to age and experience, plus USS: maximum starting salary £6,066.

Applications as soon as possible to Miss L R Heyes, Senate Registry, University of Birmingham, PO Box 363, Birmingham B15 2TT, from whom further particulars may be obtained. 3331(E)

UNIVERSITY OF FREIBURG/BR

Applications are invited for a

POSTDOCTORAL FELLOWSHIP IN NEUROBIOLOGY

for research in pharmacology of neuroendocrine functions in sleep.

Curriculum vitae and references should be sent to: Dr. H. Cramer, Professor, Section on Neurochemistry, Department of Neurology, University of Freiburg, Hansastr. 9. D-7800 Freiburg/Br., West Germany. W389(E)

UNIVERSITY OF NOTTINGHAM MEDICAL SCHOOL

DEPARTMENT OF BIOCHEMISTRY

POST-DOCTORAL RESEARCH FELLOW

Applications are invited for a research fellowship financed by an industrial company for a study of dietary phospholipids and liver lipid metabolism in relation to heart disease. The fellowship is tenable initially for one year from 1st October, 1979 or a date to be agreed. Renewal for a further two or three years is possible. Initial salary £4,333 with membership of U.S.S.

Applications with curriculum vitae and the names of two referees should be sent as soon as possible to Professor J.N. Hawthorne, Department of Biochemistry, University of Nottingham Medical School, Queen's Medical Centre, Nottingham NG7 2UH from whom further details may be obtained (telephone 0602-700111, Ext. 3488). 3235(E)

FRIEDRICH MIESCHER-INSTITUT

POSTDOCTORAL FELLOWSHIP

IN BIOCHEMISTRY

is available for the area of ß-adrenergic receptor-mediated effects in tissue culture cells. The fellowship will run for two to three years.

Candidates should send c.v. and three names of referees to Dr M. Staehelin, Friedrich Miescher-Institut, PO Box 273, CH-4002 Basel, Switzerland. W364(E)

UNIVERSITY OF LONDON

THE SCHOOL OF PHARMACY

POST-DOCTORAL RESEARCH FELLOWSHIP IN RETINAL PHARMACOLOGY

Applications are invited for a research fellowship, funded by the S.R.C. to study transmitter substances in the vertebrate retina. The appointment is for three years from 1st November 1979 or later.

Applications, stating age, academic qualifications, research experience and the names of two referees should be submitted to:— Dr M. J. Neal, Department of Pharmacology, The School of Pharmacy, 29/39 Brunswick Square, London WC1N 1AX, from whom further particulars may be obtained. 3340(E)

UNIVERSITY OF BIRMINGHAM

DEPARTMENT OF BIOCHEMISTRY

Applications are invited for an MRC-financed Postdoctoral

RESEARCH FELLOWSHIP

to work with Dr Ian P. Trayer on the role of myosin light chains in actomyosin interaction. The successful applicant will join a group investigating different aspects of the actomyosin interaction using a variety of chemical and physical techniques. The post is tenable from 1 October 1979, and is funded for three years. Starting salary will be between the points £4,333 and £4,910 (plus USS) on the Research Fellow 1A scale, depending on age and experience.

Further details can be obtained from Miss L R Heyes, Senate Registry, University of Birmingham, PO Box 363, Birmingham B15 2TT, to whom applications (2 copies), together with curriculum vitae and names of 2 referees, should be returned not later than 15 October, 1979. 3330(E)

SENIOR VISITING FELLOWSHIP PROGRAM

Centre for Interdisciplinary Studies in Chemical Physics
The University of Western Ontario
London Ontario, Canada N6A 3K7

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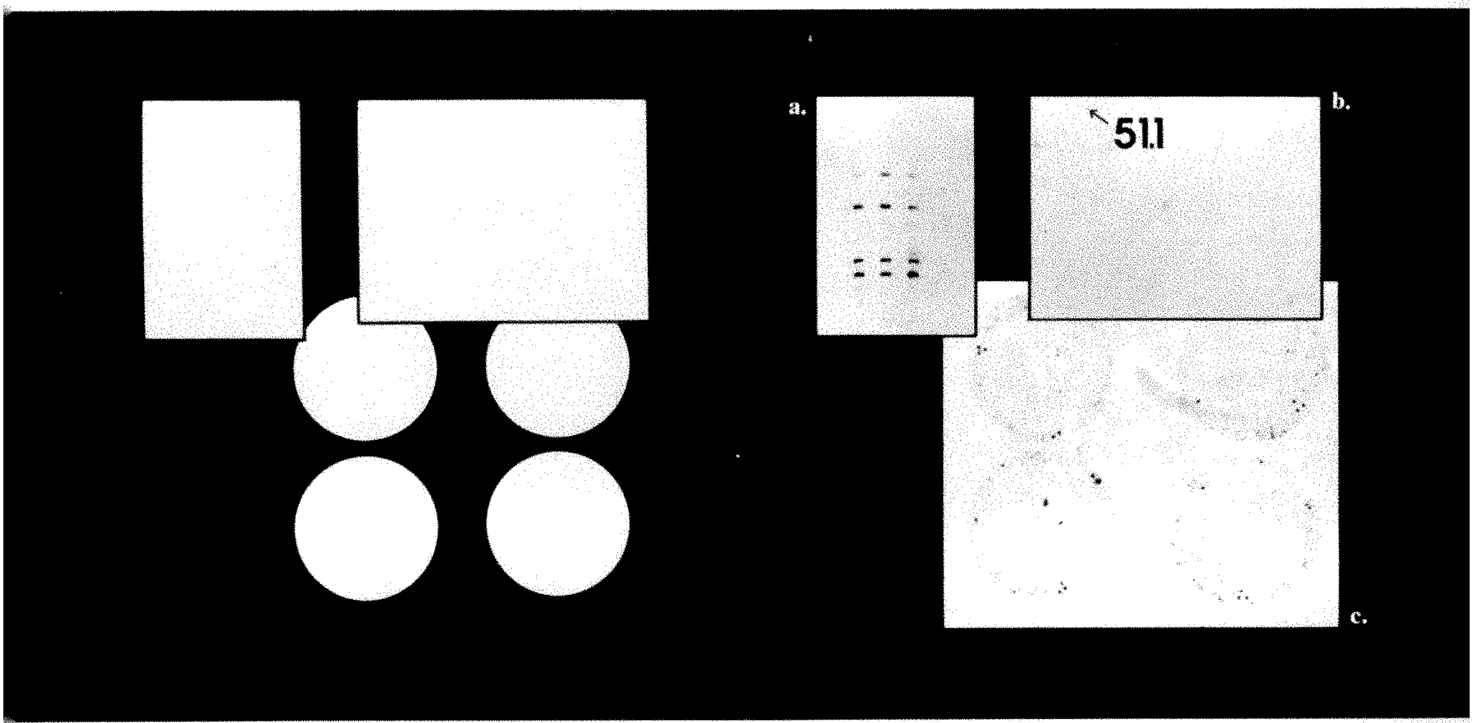

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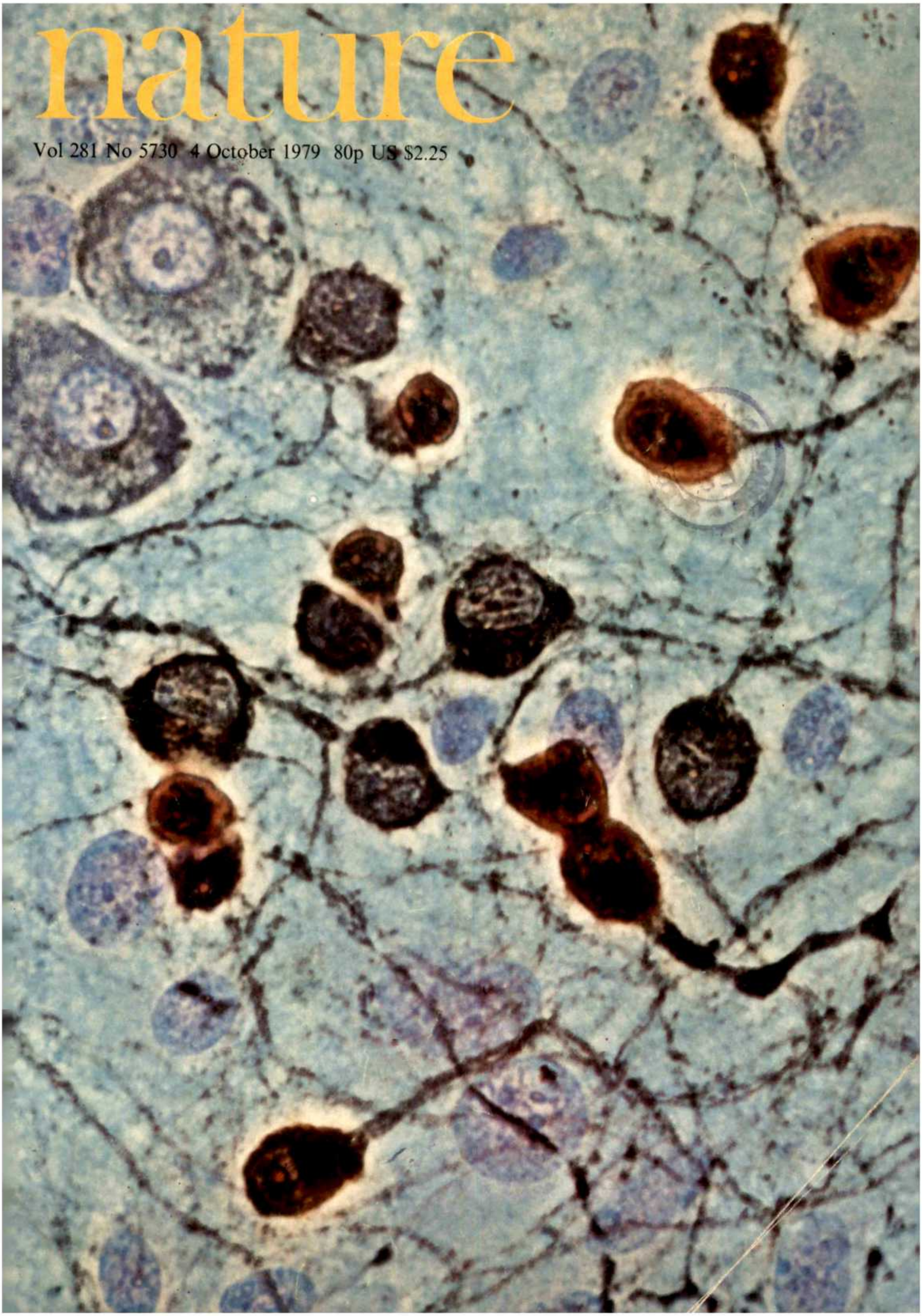

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● 'Matters Arising' permits short discussion (up to 300 words) of papers that have recently appeared in *Nature*.

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Manuscripts may be submitted either to London or New York. Three typed copies should be submitted, each including lettered copies of figures. Typing (including references) should be double spaced. The title should be brief and informative. Pages should be numbered. References, tables and figure legends should start on separate pages. Experimental detail vital to the paper yet which would interrupt the narrative is best placed in the figure legends. Units should conform to the Système International. Greek characters should be identified in the margin on their first appearance. Equations should occupy single lines if possible; exp (a) is preferred to e^a if 'a' is more than one character.

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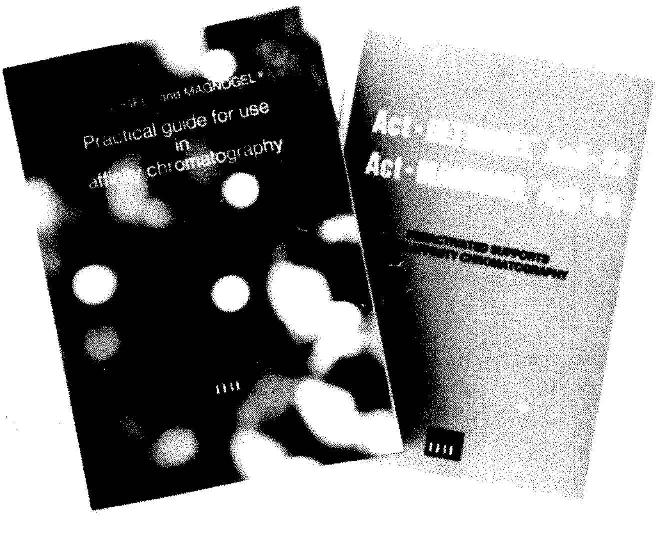

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4 October 1979

Job-swapping could relieve academic stagnation

ACADEMICS in mid-career might consider exchanging posts for a period of up to five years. Those who scan the classified advertisement columns every week are only too aware that jobs for scientists in British universities are very scarce at present. As a result of low levels of retirement, disappointing recruitment of students into the sciences, and continued financial stringency which makes every new appointment subject to the closest scrutiny, there are no more academic jobs on the market in the late 1970s than there were in the early 1960s, before the great university expansion.

The plight of those at the bottom of the academic ladder has been well publicised over the past year or two — they are finding that if they are lucky they can subsist for a few years on postdoctoral fellowships before obtaining a permanent post, but without such fortune even highly capable young people may simply have to give up any aspirations to an academic career.

Less well publicised is the problem of the person in mid-career. Remarkably few academic positions are advertised at this level, because remarkably few academics are at present vacating their posts because remarkably few academic positions are advertised because . . . . Those jobs that are freed because their holders move to professorships are largely filled internally because almost every department is experiencing heavy pressure for promotion in the ranks. The result, as is well known, is stagnation as everyone holds tight to the job they have. And stagnation ultimately leads to demoralisation.

Dr Colin Clarke, of the School of Biological Sciences in the University of East Anglia puts forward a simple proposal. He writes:— "A simple, admittedly imperfect and partial solution to this problem would be that of direct academic exchange. Thus a lecturer in, say, genetics in University A might exchange his position, and possibly his house, with a similar person in University, or even Research Institute, B with great potential advantage and stimulation to both individuals involved and their departments. At its simplest this scheme could be largely informal and need not involve more expenditure than that of mutual visits and removal costs. House exchange, rather than house purchase, might avoid further financial problems. I suggest that for both academic and family reasons there would need to be a minimum period for such exchange, of perhaps 3 or 5 years. A permanent, or longer term, exchange could then follow, but only if both partners and both departments were satisfied with the arrangement.

"This scheme has the advantage that it allows for the exchange of people whose scientific interests are not exactly matched, in some cases to the clear advantage of particular pairs of individuals or departments. Thus someone working in, let us say, applied plant genetics might exchange with another person whose interests are recombination in bacteria, depending on the existing interests and staffing of the two departments involved.

"The above scheme might operate via a personal advertisements section in *Nature*. Below is a sample advertisement, of the type envisaged, which I have written for myself. There will, of course, be objections to this scheme, not least perhaps from universities if the persons contemplating exchange are not on identical salaries. The present national academic situation is, however, so stagnant that solutions of the above type are highly desirable and their advantages to individuals and departments far outweigh administrative objections.

EXCHANGE SOUGHT

Reader in Microbiology, University of East Anglia, Norwich, research interests in Microbial Mutagenesis and Bacterial Morphogenesis, general interests in many aspects of General Microbiology and Microbial Genetics. Extensive teaching experience mainly Microbiology and Genetics. Exchange over 5-year, or longer, period for Sept 1982 onwards with like person in University or Research Institute, perhaps in Midlands or Scotland. Please contact Dr Colin Clarke, School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ (Phone 0603-56161, extn. 2232). It may be possible to arrange a housing exchange involving 4-bedroom, detached house with garage and gas c.h. in Norwich.

Would it work? Dr Clarke will be able to tell us fairly soon. The record of the civil service, which tried out a similar mobility scheme a few years ago, is no encouragement. Interest in temporary exchanges was insufficient to warrant continuing the scheme after a year or two. But maybe academics are different. And maybe there is a growing awareness of the importance of sideways moves. If so, then *Nature* will be glad to support such a scheme. □

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Carter faces new dilemmas over non-proliferation

In the last of three articles about US attempts to regulate the social effects of science and technology, **David Dickson** describes the problems raised by President Carter's policy for controlling the spread of nuclear weapons

ONE of the main issues in the US presidential elections next year — just as it was when Jimmy Carter faced Gerald Ford three years ago — is likely to be whether the US should revise its attitude towards the relationship between the spread of nuclear energy and the proliferation of nuclear weapons.

Three years ago Carter gained considerable political capital from his firm stand against both the reprocessing of spent fuel and the development of fast breeder reactors, arguing that if not properly controlled, both could increase the risks of nuclear weapons proliferation — by encouraging production of weapons-usable plutonium. A week before the election he castigated President Ford's initiatives in the same direction as "too little and too late".

Now it is President Carter's own policies that are on trial. In particular attention has focussed on the results of the Nuclear Non-Proliferation Act (NNPA) passed overwhelmingly by Congress last year, and whether the strategy embodied in this act — attempting to control weapons proliferation through abrogation of commercial reprocessing and unilateral restrictions on uranium supply — remains the most appropriate direction to take.

Some argue that the apparent failure of the US to achieve a significant consensus in support of this strategy at the international level indicates inherent flaws. They point in particular to the provisional conclusions of the International Nuclear Fuel Cycle Evaluation (INFCE), set up at Carter's suggestion in 1977 and due to report formally next February, which are expected to give little support for the US strategy.

Others argue, conversely, that the administration has not been strict enough in applying its restrictions. "Many of us feel that there has been a great deal of back-tracking by the administration, and that this seems to be accelerating rather than slowing down" said one Congressional aide last week, pointing for example to delays in implementing certain aspects of the act and the administration's apparent leniency in accepting conditions for the retransfer of spent nuclear fuels for reprocessing.

Three aspects of the proliferation issue are likely ot rekindle a public debate which has been relatively dormant since the signing of the NNPA last spring. The first is the failure of the US, despite cutting off economic and military aid, to dissuade Pakistan from what many believe to be its current attempts to develop nuclear weapons. "If Pakistan does explode a nuclear device, then it could have the same galvanising effect as Three Mile Island did," predicts one observer.

The second aspect is the growing economic crisis of the US nuclear power industry. At present the industry is still meeting past orders; but domestic orders have virtually dried up, and export orders may be the only way to keep the industry alive. But these are now seriously hampered by US non-proliferation policy.

The third factor affecting the debate will be the occurence of a number of international events focussing on the proliferation issue, including the domestic debate on a comprehensive test ban treaty (hopefully agreed in draft form by the US, USSR and UK in the near future), the review of the Non-Proliferation Treaty signed in 1970, and the outcome to the INFCE discussions.

INFCE: technical or economic?

Despite a relative lack of support for the thesis that the threat of proliferation requires of itself a reduced commitment to reprocessing and fast breeders (even though this may result *de facto* from technical and economic problems), US officials claim that the INFCE exercise has been a success in getting the link between plutonium production and the threat of proliferation firmly on the international agenda.

"INFCE has accomplished the largest part of what it was meant to do. As well as making a number of specific suggestions on way to go forward, it has been a process of international education, and despite difference between individual countries, there has been a considerable convergence over the past two years", says Dr Nye, one of the chief architects of the act and now Professor of Government at Harvard University.

However INFCE is unlikely to result in any major international shift towards the position so firmly enunciated by President Carter three years ago. (Indeed some suggest that the study may have been partly arranged to get the President off the hook). And despite the US administration's insistence that it was always intended as a technical exercise, and not a negotiating forum, scepticism of the US stance is based on the concern that technical exercises have been used as a front for political and economic interests.

Many European countries, for example, have accused the US of using its anti-reprocessing stand as a means of exploiting its market potential as a major supplier of enriched uranium, and of making up for its lack of progress in fast breeder design.

Political distrust also lies behind the rejection of US non-proliferation policies by Third World countries such as India and Pakistan. These countries see attempts by the US to prevent their access to nuclear technology, on any grounds, as an illegitimate infringement of sovereignty.

Domestically US politicians show little embarrassment for arguing that the US should use its position of nuclear hegemony to both articulate and justify its strategy on proliferation policy. "Unfortunately, certain countries have refused to see the wisdom of our position. If this situation persists, they may force the US to discontinue further nuclear collaboration," Representative Jonathan Bingham, one of the staunchest supporters of the NNPA, said recently.

At the same time, administration officials are increasingly concerned that the US position on non-proliferation strategy may be undermining the US's hegemony on nuclear issues in favour of various European countries — and that this could have wide economic and political implications.

Last week, for example, the Senate rejected a motion to discontinue research on the liquid metal fast breeder reactor at Clinch River in Tennesee (a long-time target of President Carter's). The main arguments for rejection were couched no so much in terms of its technical and economic appropriateness, severely questioned by the administration, but on the argument that the US is several years behind Europe in fast reactor design, and that this alone was sufficient reason for pressing ahead with the project.

Given the distrust that exists both in Europe and in the Third World over the political implications of the US non-proliferation strategy, one of the few statements that can be made with certainty is that international consensus is still a long way off — and that reaching such a consensus will be one of the major diplomatic tasks of the 1980s.

One major source of contention during the INFCE discussions, for example, has been on reaching agreement on the extent of global uranium supplies. Those opposed to the urgent need for reprocessing have argued, in support of their position, that global supplies are plentiful; those supporting both reprocessing and fast breeders support a much lower estimate, adding weight to their position.

The resulting assessment of supplies is likely to be an ambiguous compromise between these two positions, with a range of possible estimates. "The vocabulary may be largely technical, but the messages and stakes are highly political, and as such INFCE presents a fascinating case study in the interaction of science and politics,"

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Canisters of uranium-238 stockpiled at Paducah, Kentucky — the 'spent fuel' that can be converted to plutonium in a fast breeder

commented Peter Clausen of the Department of Energy's Office of Energy Research in a recent issue of *Arms Control Today*.

Domestically, the provisions of the Nuclear Non-Proliferation Act have placed new demands on the agencies required to see that they are carried out — in particular the Department of State, the Department of Energy, and the Nuclear Regulatory Commission.

The State Department, for example, has had to bear the brunt of external criticism of the administration's policies, particularly from countries with which it is actively seeking cooperation in other spheres; some critics claim that this has tempted the department to make compromises in carrying out its role — for example, in allowing reprocessing for the purposes of breeder research — that go considerably beyond Congress' original intentions.

The role of the Department of Energy, long an ally of the very industry which has been most affected by the NNPA, has also come under fire, critics claiming a conflict of interest between its functions of promoter and regulator of enriched uranium exports.

Finally several members of the Nuclear Regulatory Commission have expressed public disquiet about being asked to judge the adequacy of safeguards in countries which made requests for enriched uranium. This required the commission to play a direct foreign policy role for which it is ill-equipped.

"It is a major foreign policy action for an agency of the United States Government to go to a foreign country and say: we must inspect your facilities, we must inspect your system, before we will grant an export license. The NRC staff are not foreign policy experts," Commissioner John F Ahearne said in an address "Does the Emperor Have any Clothes?" given in New Orleans three weeks ago.

Government agencies, however, are not the only ones having difficulty with grappling with the issues thrown up by the debate proliferation. There are also deep differences in strategy among environmental groups concerned with the dangers of nuclear power in all its forms.

Some argue that the prime need is to define the technical parameters of the licensing process in a way that minimises the environmental impact of nuclear power. "We have tried to get political considerations out of export licensing," says Jacob Scherr of the Natural Resources Defense Council (NRDC), arguing against Commissioner Ahearne that it is possible to define criteria for the adequacy of safeguards that are "technical and objective".

Others, however, argue the case against nuclear technology on the more explicitly political grounds of opposition to the social and economic interests which it supports. "The NNPA is more a mechanism for sharing the nuclear market than an attempt to curtain nuclear weapons in the developing countries; certainly it is looked upon by these countries very cynically as a market control mechanism," says Bob Alvarez of the Environmental Policy Centre (EPC).

Such different perspectives can lead to a considerable divergence in policy. A particular case is over whether or not the US should fulfil its commitment to provide storage facilities for fuel which has been used on foreign reactors. Groups such as the NRDC argue with the administration that providing such storage, coupled with a guarantee of providing enriched uranium fuels, will take the pressure off the need for reprocessing facilities in foreign countries — and hence help reduce the chances of weapons proliferation.

In contrast, EPC and other nuclear groups argue that the US's provision of storage facilities would not only present a potential hazard to the US environment, but would also help encourage the spread of nuclear energy by getting other countries off the hook on the question of waste disposal. "Our philosophy is more concerned with restricting the spread of nuclear technology itself, rather than using a safeguard approach based on restricting the supply of nuclear materials," says Dr David Berrick of EPC.

Somewhat less predictably, the proliferation issue has also recently raised some difficult constitutional questions. These result from the recent publication of an article giving scientific and technical details of the construction of the hydrogen bomb, all claimed to be derived from public, non-classified information.

Finally there is the thorny question of how to deal with the Non-Proliferation Treaty when it comes up for review next year. At one level there is the question of the extent to which recent moves by the nuclear countries to restrict the export of "sensitive technologies" contravene Title IV of the NPT, which promises "the fullest possible exchange of equipment, materials and scientific and technological information for the peaceful uses of nuclear energy."

Perhaps more fundamental is the fact that,as it currently stands, the treaty codifies an inherent inequality between weapons states and non-weapons states. This condition that may have appeared appropriate when the treaty was negotiated in the late 1960s; but it fits uneasily with the demands made for a new international economic order, implying a form of North-South hegemony which many countries have demonstrated their unwillingness to accept by refusing to sign the treaty.

In the short-term, the Carter administration is now faced with the prospect of Pakistan exploding a nuclear device, and thus joining the "nuclear club" within the relatively near future. A state department study group was set up under the direction of Mr Smith late in August to discuss possible strategies by the US to prevent this, given that its current attempts to apply pressure through sanctions seem to have failed. And already some are suggesting that these sanctions should be stiffened.

In the long-run, there is the issue of whether a sufficient consensus — involving both developed and developing countries as partners in decision-making — can be forged to develop and apply an effective control regime; or whether increasing competition between both producers and consumers of nuclear power will be such as to limit the possibilities for multilateral action, shifting the focus back to bilateral actions. At this stage, nobody pretends to have all the answers. □

US adopts new cancer policy

NEW guidelines setting out a common framework for both the scientific assessment of potential carcinogens and the appropriate regulatory mechanisms were announced by the US Government last week. The guidelines, described as a "Government-wide cancer policy", were published by the regulatory council set up by President Carter last October to coordinate federal regulations in general. (See *Nature* 27 September 1979 p244).

On the scientific side, the council supports that view that animal tests are a valid method of determining whether a substance should be considered a potential carcinogen. It also says that a single animal test, if properly conducted, is sufficient to indicate "a potential risk to humans" for regulatory purposes — a view contested by the chemical industry in current debates over the proposals of the Occupational Safety and Health Administration to establish a "generic carcinogen policy".

Referring to claims that a single positive test is not enough, the council says that "although the agencies should attempt to obtain additional data, they should not take the risk involved inwaiting two to four years" to conduct further tests before taking action. At the same time, the council has gone some way to meeting industry's demands by caling on agencies to ensure that proposals for regulating carcinogens are accompanied by some form of risk assessment, including in particular determining how many people are exposed to the substance.

The council implies that economic considerations should be included, urging agencies to identify and consider the least costly and least disruptive course of action in planning cancer controls. It also says that agencies should consider "zero risk" bans in cases where the economic and social costs are considered minimal.

"This new policy will ensure that the various agencies will operate from the same scientific base in determining whether a compound is carcinogenic", Mr Doug Costle, chairman of the Regulatory Council and head of the Environmental Protection Agency, said in announcing the new guidelines.

David Dickson

School for scandal?

Soviet University admissions procedures are being circumvented by over-ambitious parents, *Pravda* alleged recently. The offenders, it claimed, are making use of their professional position or contacts to obtain their children a priority position on the placement lists.

Admission to Soviet Universities and other higher educational institutions is by competitive examination. However, even for those who have passed the examination, there may still not be enough places. Priority is therefore given to "front-rankers" from agriculture or industry, who are recommended and supported by their former place of work, to which it is assumed they will return.

This priority scheme is intended to provide Soviet production with the specialists it needs where and when it needs them. Modifications are made from time to time to meet specific demands. Thus the normal length of service needed for a "front-ranker's" place is two years. However, in April of this year, workers sponsored by the Ministry of Land Reclamation and Water Conservancy were exempted from any minimum length of service presumably due to pressing demands for graduates in that sector.

Although no less than four signatures and an official seal are needed on the "front-ranker's" sponsoring form, "crafty parents" says *Pravda,* are managing to equip their sons and daughters with false papers. Not surprisingly, those who graduate under such auspices are in no hurry to start working at the farms or enterprises which sponsored them. Not surprisingly, too, a number of the alleged "front rankers" have been abondoned in mid-course by their "sponsors" and have had to apply for state maintenance grants. Last July, the Central Committee of the Communist Party of the Soviet Union and the USSR Council of Ministers adopted a new decree on the "Further Development of Higher Education and Raising the Standard of Training of Specialist".

"Before you go in, stick this chicken under your arm and put some manure on your boots!"

This provides for a new procedure of job allocation. By fixing a student's future job some two or three years before he or she graduates, the "output" of young scientists and technologists will, under the new system, become part of the normal Five Year Plan. Moreover, and particularly in the Far North and Siberia, measures are envisaged to retain the graduates in the area on a permanent basis.

Parents about to furnish their children with the certificates of Arctic lumberjacks would be well advised to think twice.

Vera Rich

Superpower clash over test ban technology

Delegates to the Geneva talks on a comprehensive test ban, currently nearing completion after almost two decades of negotiation, have reached an impasse over USSR demands that only Soviet equipment be used for the seismic monitoring on Soviet soil.

Until three months ago the US had assumed that the Soviet Union would permit the use of American detectors on its soil. Data from the detectors will be used to verify that seismic disturbances are the result of natural causes and not the undergound testing of nuclear weapons banned under the terms of the treaty.

However in June members of a US Congressional delegation visiting Moscow were told by Soviet officials that they would only permit Russian equipment to be used for this purpose — a condition which, in the eyes of the US politicians, would make the treaty unacceptable.

"We feel that it is essential to use US equipment to verify Soviet compliance; and that this point is so central that we cannot allow the test ban talks to proceed unless this is resolved," an aide to Representative Jack Kemp (Rep, New York), said recently.

State Department officials in Washington have been playing down the significance of the USSR demands, claiming that they see them as essentially a negotiating tactic which will be dropped in return for some concession from the US side. They have already rejected a request from Mr Kemp to cancel a visit by Soviet scientists last month to study US seismic technology — a visit which Mr Kemp described as "scientific espionage".

Furthermore seismic experts in the US feel the USSR would have little to gain from insisting on the use of their own equipment, since the US already possesses suitable equipment that can be bought "off the shelf"; existing Russian equipment, it is claimed, lacks either the dynamic range or the precision of US seismic sensors.

Others involved in the negotiations, however, maintain that although relatively trivial, the issue is the sort which could seriously delay completion of the treaty, since no quick way out of the impasse can currently be seen. Any such delay could also effect the outcome of the second Non-proliferation Treaty Review Conference, which takes place next spring, and at which the non-nuclear powers will demand evidence from the nuclear powers of steps which have been taken to prevent escalation of the nuclear arms race.

Congressional aides said in Washington last week that they expect the issue of Soviet technology to be raised dring hearings on progress towards the test ban treaty due to be held by the House Armed Services Committee later in the autumn. ☐

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news in brief

"Employers need freedom": Mr Mark Carlisle, UK Secretary of State for Education and Science has refused to establish an enquiry into the problems of short term contract researchers. In a letter to the Association of Researchers in Medical Sciences (ARMS) the minister's private secretary transmitted Carlisle's sympathy for those on short term contracts and his reluctance to see "good research wasted because it had to be ended prematurely". But, in accordance with current Conservative philosophy, Carlisle affirmed the importance of the employer in economic development. "On the other hand, the effective development of research is liable to be hampered unless employers have sufficient freedom to manoeuvre." An ARMS spokesperson said the government's response was "dismal."

Piperno hearing postponed: Extradition hearings in Paris for Franco Piperno, the Italian physicist accused of anti-government activities (27 September) have been postponed until 27 October. In the meantime, the French civil liberties organisation CINEL (Centre d'initiative pour des nouveaux éspaces de liberté) has raised questions about the prison conditions of Piperno and another detainee, Lanfranco Pace. In Italy 60 Milan lawyers have issued a statement opposing the extradition. The lawyer's statement accuses the Italian authorities of not wanting a true public inquiry but instead "to submit (Piperno and Pace) to a long preventative detention in special well known prisons."

India-USSR to collaborate on solar energy: An Indian team of scientists which visited USSR in July has signed an agreement to collaborate with the USSR on a programme of solar energy utilisation. The programme invisages the development and testing of selective surfaces and solar collectors, solar thermoelectric generators and an autonomous power station using a Stirling engine. The joint work will be carried out at Central Salt and Marine Chemicals Research Institute, Bhavnagar; National Physical Laboratory, New Delhi; Kryzhizhanovsky Power Engineering Institute, Moscow; and the Institute of Physical Studies, Tashkent. *From Zaka Imam, New Delhi.*

New Finnish nuclear incident: Following recent difficulties with the Soviet built Loviisa nuclear power station, a radioactive leak occured at the Olkiluoto-1 station, which is of Swedish origin. The incident on 29 August took place when the station was working at 65% capacity. A pipe in the reactor water purification circuit burst, and some 5 m^3 of radioactive water escaped on to the floor. All safety systems worked satisfactorily, and, according to the Teollissuden Voima (Industrial Power) Company which owns the station, the leak was immediately isolated and the station shut-down. After tests by the Radiation Protection Institute, the station was allowed to recommence operation on 3 September. The shut-down had no effect on Finland's plans for nuclear energy expansion. While Olkiluoto-1 was still out of action, the Council of States granted an operating licence of Olkiluoto-2 (subject to final checks). This second set is expected to commence generating at the beginning of 1980 and to reach full capacity by the middle of the year.

More Windscale troubles: Thirty people had to be evacuated from a low level effluent treatment plant on 12 September when radiation alarms sounded in the early morning hours. Plutonium was part of the radioactive airborn release but the details on levels or amounts is not known. A British Nuclear Fuels spokesperson said that tests have shown "no signs of harmful contamination". The cause of the alarm and the source of the leak are the subject of an internal inquiry. In the meantime the inquiries into the fire in the decanning cave last July in which 8 workers suffered radiation exposure (26 July) and the sump leak last March that discharged 30,000 curies under a temporary storage building (26 April) are "still proceeding."

IEA launches International Energy Conservation Month: International Energy Conservation Month for the 20 members of the International Energy Agency began on Monday. To increase consumer awareness of the need for energy conservation, the IEA member states are organising 30 international conferences and exhibitions to take place during October in different cities throughout the world, as well as individual national events including local meetings, exhibitions, demonstrations and competitions for school children. In addition, two countries, the US and Japan, are using the month to launch new legislation on energy conservation. President Carter, for example, is expected to announce the enforcement of the 55 mph speed limit and the issue of a gas mileage guide.

Plant physiologist expected to be named head of new US research foundation: Dr Nyle Brady, currently director of the International Rice Research Institute in the Phillipines, is expected to be nominated by President Carter as the director of the new Institute for Scientific and Technical Co-operation (ISTC), which will become the focus of US research efforts on issues directly related to the needs of developing countries. Two of Dr Brady's principal research interests have been the fundamental effects of lime in soil, and the physiology of the peanut plant (an interest which White House officials deny had any bearing on his appointment). Other research interests include the influence of fertiliser on the yield of rice, corn and coffee, and the influence of soil temperature on nutrient uptake.

Meanwhile the Senate appropriations subcommittee last week approved an allocation of $19.75 million to the ISTC for the fiscal year beginning 1 October 1979, in addition to funds transferred from programmes currently under the Agency for International Development. However the institute's budget figure still has to be approved by the full Senate appropriations commitee and by the Senate itself — at both of which stages it is expected to meet with opposition — before the institute can be assured that it will be allowed to start functioning.

US agrees to increase research cooperation with Japan: The United States and Japan have agreed to increase cooperation on scientific and technological research into non-energy fields, a move which follows an earlier agreement to cooperate on energy research. The broad framework for cooperation was agreed at a meeting in Tokyo last week between as US delegation headed by Dr Frank Press, director of the Office of Science and Technology Policy, and a Japanese delegation headed by Mr Moyazaki, deputy minister for foreign affairs.

Following summit talks held last May, a communique was issued stating that the two governments "will study seriously the prospects for cooperative efforts in areas of basic and applied research", adding that promotion of joint cooperation was important in building up a partnership between the two countries for the 1980s.

No details have yet been announced of specific areas for joint research activities. However possibilities include, outer space development, and environmental and health research.

Bombay magnetic observatory may move: The Colaba-Alibag Institute of Geomagnetism, with its 133-year-old record of continuous magnetic observation, may be moved if electrical noise from a nearby fertiliser complex disrupts its function. A campaign (*Nature* 15 February) to have the fertiliser complex at Thal-Vaishet resited has failed, in part because the state government of Mararashta opposed the move because of the loss of jobs resiting would have entailed.

A number of experiments on the effects of electromagnetic noise have so far proved inconclusive, however, and there is still the possibility that the observations will be unaffected by the plant's machinery.

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Burying Britain's radioactive waste

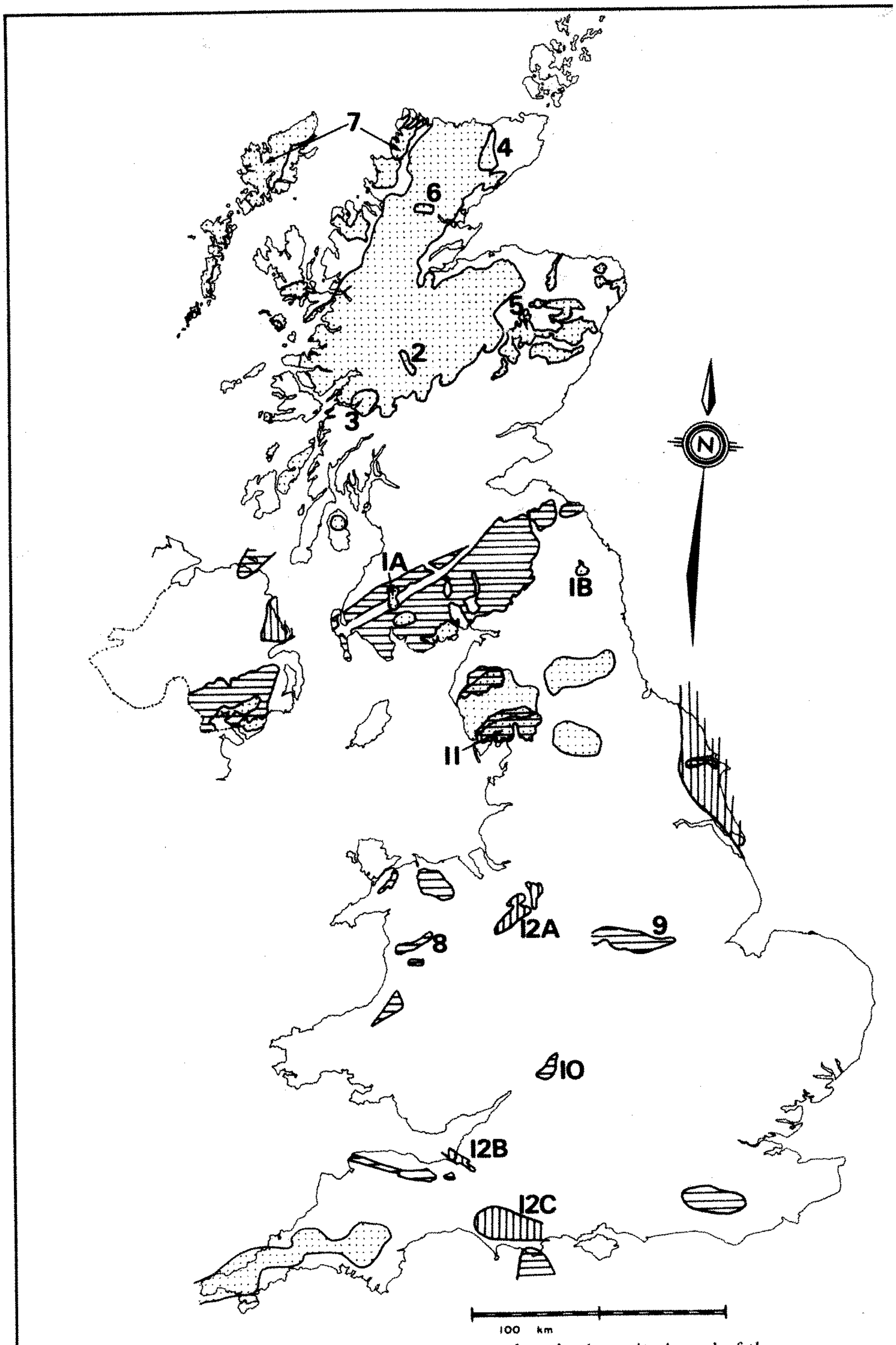
— the geological areas under investigation

By J D Mather, D A Greenwood and P B Greenwood of the Institute of Geological Sciences

OVER the next decade a major research effort is planned in the United Kingdom into the feasibility of the disposal of solidified, highly-radioactive, heat-emitting wastes to geological formations. Such research was called for in the Sixth Report of the Royal Commission on Environmental Pollution published in 1976[1] and accepted as one of three possible disposal options in the Government's White Paper[2] of 1977. These are disposal:

- on the bed of the deep ocean,
- into stable geological formations on land, and
- disposal under the bed of the ocean.

Parallel studies into all three options in relation to high-level wastes are being undertaken but the land option has progressed rather further than the oceanic programmes. The White Paper recognised that the Natural Environment Research Council's Institutes of Geological and Oceanographic Sciences will play an important part in this research and relevant programmes are now under way at both Institutes. Criteria for use in the selection of areas under land which contain geological formations potentially suitable for such disposal in the United Kingdom were published in 1976 by a Working Party established by the Council's Institute of Geological Sciences[3]. A recent comparison of the criteria with those published elsewhere has indicated a high degree of international agreement, so that no significant revisions are necessary[4].



Map showing the distribution of rocks thought to meet the selection criteria and of those areas which have been selected for feasibility studies. Crystalline igneous and metamorphic rocks are stippled, argillaceous rocks are shown with horizontal lines and evaporites with vertical lines. The crystalline rocks include the postulated subsurface outcrops of the granites of northern and south-western England. No rock types meeting the criteria occur in the Shetland Islands.

The principal objective of disposal of these solidified wastes into geological formations is to ensure that they are placed in a situation where the radionuclides cannot be returned to the biosphere in amounts constituting a biological hazard. In reality that means placing them in a repository constructed at depth in a geological formation or rock mass able to withstand the effects of decay heat and radiolysis and sufficiently free form circulating groundwater to ensure their containment[5,6]. The programme is planned in a series of stages of which the present proposals relate to the exploration phase, during which no radioactive wastes will be used. Some sections of the UK national research programmes are being mounted jointly with the Commission of the European Communities.

The application of the geological criteria results in the recognition of 127 different areas containing potentially suitable host rocks. These range in area from less than 5 km^2 to around 6,000 km^2, the mean being around 260 km^2. Of these areas, 104 contain crystalline igneous or metamorphic rocks, 17 argillaceous rocks (fine grained sediments consisting principally of clay minerals) and six evaporites, including hybrid evaporite/clastic sequences. The distribution of the areas involved is shown in the map above and covers nearly 39,000 km^2, approximately 16% of the land area of the UK.

In order to select representative areas for feasibility studies a further desk study was undertaken using as primary factors the geological environment and the evolutionary history of the rocks concerned, so far as they are known. The groups thus obtained were then further

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Areas for further study

Crystalline rocks

1. Caledonian granites intruded into (1A) Lower Palaeozoic sediments or (1B) volcanic rocks. The Loch Doon Granite (Intrusion 1A) is a zoned body with an outer unit of biotite tonalite merging progressively into a central unit of biotite granite. It is intruded into Ordovician sediments of various types, predominantly greywackes, but with significant developments of black shales and cherts and has a sharp contact with an extensive thermal aureole. The Cheviot Granite (Intrusion 1B) varies in composition from a pyroxene granite to a pyroxene-free granophyre, is intruded into andesite lavas and has a faulted brecciated contact.

2. Caledonian granite forcefully intruded into Pre-Cambrian metamorphic rocks. The Ossian Granite is a coarse hornblende-biotite tonalite varying to granodiorite towards the outer part. A number of SW-NE trending fractures cross the granite which is intruded into metamorphosed sediments belonging mainly to the Pre-Cambrian Moine Supergroup. No thermal aureole is developed but the intrusion margin is generally intimately welded to the country rock by the development of contact migmatites.

3. Caledonian granite permissively intruded into Pre-Cambrian metamorphic rocks. The Etive Complex is a ring complex composed of 4 successive granite intrusions emplaced as a result of progressive cauldron subsidences. The innermost unit known as the Starav Granite is a quartz adamellite which has a simple, near-vertical contact and is free of dykes.

4. Granitic complex in the core of a regional migmatite zone. The Strath Halladale Granite occupies the core of an injection complex within metasediments of the Moine Supergroup. The injected material is dominantly biotite grantite of variable grain size which occurs as veins or concordant sheets within the psammitic host.

5. Caledonian basic intrusive. The Morven/Cabrach Intrusion is mainly composed of norites with associated gabbros and diorites. It is steepsided with steeper dips on the west than on the east, implying an increase in width at depth. Much of the intrusion is extensively altered with pyroxene replaced by amphibole.

6. Non-migmatised Pre-cambrian Moine Metasediments. The rocks of the Moine Supergroup consist of thick metamorphosed arenaceous and argillaceous sediments with some intermediate types. The area around Inveroykell is being considered within a region of massive to flaggy quartzo-feldspathic psammi of intermediate metamorphic grade and relative lack of structural complexity.

7. Pre-Cambrian Lewisian basement rocks. The Lewisian includes relics of several orogenic cycles covering a time span of at least 2,800 my to the latest reworking at 1,600 my. The major sub-division is into two time-based complexes, the older Scourian and the younger Laxfordian. The Laxfordian rocks are dominantly grey quartzo-feldspathic and biotite hornblende gneisses with intrusive sheets and veins of granite and pegmatite. The gneisses have undergone thorough re-working resulting in rock units that are relatively homogeneous compared with much of the rest of the Lewisian basement and appear the most suitable as a disposal medium. Such rocks occur in North West Scotland, between Loch Laxford and Cape Wrath and in the Outer Isles, but so far no specific research area has been identified.

Argillaceous and evaporite formations

8. A series of gently folded Ordovician and Silurian rocks in North Wales dominated by argillaceous types totalling some 4,000 m in thickness. The rocks are well exposed at the surface and dip consistently to the south-east at an angle of about 45°.

9. A sequence of turbiditic mudstones with interbedded siltstones and limestones of Lower Carboniferous age. The rocks occur within a fault bonded trough known as the Widmerpool Gulf and a minimum thickness of 1,000 m of argillaceous rocks is available. These rocks constitute a good example of the effective compromise desirable in argillaceous rocks — a thick formation that has been dewatered and developed a mature clay mineralogy by deep burial, without the attendant development of structural complexity.

10. A mudstone dominated sequence of Triassic rocks attaining thicknesses just in excess of 500 m in the central part of the Worcester Basin. These occur within the evaporite-free part of the major depositional basin with a cover of up to 500 m of mainly argillaceous rocks of Jurassic age.

11. A series of Silurian mudstones and greywackes in Southern Cumbria with an overall thickness of around 5,000 m. These rocks are folded on both a large and small scale and are cleaved to a lesser or greater extent depending on lithological variations.

12. Hybrid evaporite/mudstone sequences within Permo-Triassic sediments developed in the Cheshire (12A), Somerset (12B) and Wessex Basins (12C). These are all major depositional basins where the rocks of interest are in excess of 600 m in thickness and are generally overlain by other argillaceous formations. In all these areas examination of the cyclicity of the hybrid sequence from the point of view of waste containment is a particularly important aspect.

divided on the basis of compositional differences, degree of consolidation (for argillaceous rocks), structural complexity and other aspects to produce a tentative order of preference based entirely on geological factors. It must be emphasised that this order of preference is necessarily based on subjective judgements; if the data were available for an objective analysis there would be no need for the present research programme.

As the programme advances and more data become available it can be expected that some areas will be rejected and others introduced. It should also be noted that the areas selected relate to high-level wastes and that other formations in additional areas may be relevant to the disposal of intermediate and low-level wastes.

Crystalline rocks

Using the order of preference, 23 crystalline rock areas were selected for more detailed investigations, including reconnaissance field visits where possible. These 23 areas included a wide range of geological environments test the validity of the aproach on which the grouping had been based. A combination of those studies and selection based on the non-geological factors of land ownership and access to drilling sites resulted in selection of eight areas within seven different geological environments for detailed research into the feasibility of geological disposal of high level wastes into crystalline metamorphic and igneous rocks. Detailed analysis is required to relate the primary factor, groundwater flow, to rock mass and rock material parameters, such as mineralogy and petrology, joint frequency, attitude and openness, and regional stresses. A data base can then be considered so that the most appropriate crystalline environments for consideration as repositories can be defined.

The geological environments chosen for the future feasibility studies, and the individual areas concerned, are shown in the map and a brief description is given alongside.

Before work proceeds at any of the prospective research sites applications have to be submitted under the planning regulations for permission to drill investigatory boreholes. So far such applications have been submitted for sites within the Loch Doon Granite (Area 1A), the Cheviot Granite (Area 1B) and the Strath Halladale Granite (Area 4). Only the latter has been granted and the others are at present the subject of appeals. The research site within the Strath Halladale Granite occupies some 100 km^2 within the Caithness District and is peat and drift covered with only limited exposure of bedrock.

A preliminary drilling programme has been undertaken to confirm the limited geological information previously available and three boreholes have been drilled to 300 m, together with 24 boreholes to some 40 m. The data obtained from the boreholes complemented by field mapping, surface and borehole geophysics and detailed hydrogeological measurements are being used in a preliminary review of the containment characteristics of this particular geological environment. Similar studies are planned for the other research sites in the crystalline, argillaceous and evaporitic host rocks.

Argillaceous rocks and evaporites

The approach used in the studies of the feasibility of geological disposal to argillaceous rocks and evaporites is broadly comparable to that used for the crystall line rocks and total of seven preferred areas have been selected. The geological factor particularly relevant to the selection of these areas is the predictability of their mass characterstics. Impersistent developments of minor lithological variations may not pose problems but the presence of, for instance, a thin calcareous sandstone within an otherwise uniform greywacke/siltstone sequence, could be of major significance from the viewpoint of waste containment.

A useful property of plastic clays is their potential capacity for self-sealing. With progressive compaction and burial this property will be lost but will be countered by an increase in thermal stability as a result of the expulsion on non-structurally held water. As these changes are mutually exclusive an intermediate compromise has generally been sought involving for instance selection of areas containing uncleaved mudstones.

Although salt domes occur beneath the UK Continental Shelf they appear to be absent beneath the land mass and the evaporite formations available are all bedded deposits. These generally occur within thicker argillaceous formations and are gybrid sequences containing interbedded evaporites and mudstones and/or siltstones.

The areas containing argillaceous and evaporite formations which have been selected provisionally for more detailed study are also shown in the figure and briefly described in the box.

It is hoped to mount studies to examine the feasibility of geological disposal to argillaceous rocks at sites in each of areas 8-11 and at one site within areas 12A-12C for preliminary studies on the hybrid evaporite/mudstone sequences. Work has not advanced as far as with the crystalline rock studies and at the present time no research sites have been identified and no applications for permission to drill boreholes have been submitted.

Conclusions

The feasibility of the disposal of high-level radioactive wastes within the geological framework of the United Kingdom still remains to be demonstrated. The immediate need is for comprehensive site-specific field data from a range of potentially appropriate geological environments both to test the criteria on which these environments were chosen and to provide a data base from which potential disposal sites may be identified. The feasibility studies suggested represent a major step towards these objectives and need to be initiated as a matter of urgency.

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Sun-soaked but blind to solar power

ON the counters of the Caixa General de Depositos, the largest government bank in Portugal, bright orange folders decorated with a smiling Sun attract everyone's attention. They announce credit facilities for equipping private homes with solar water heaters. Up to £500 loans can be obtained at 10% interest over 10 years, an extraordinary bargain in a country where inflation reaches 50% a year and interest rates on short term deposits are 20%. "The response, however, has been minuscule" says a bank official. "There is no echo of interest in the public; people just don't know what it is."

The first installation financed by the Caixa was hastily inaugurated on June 23, a Saturday christened Sun Day, in a village 20 miles south of Lisbon. Here a successful architect has equipped his new country home with three solar panels in the garden. This 300 litre system cost £800. The installer, Mr Falconer, is a British subject and one of the two pioneers of solar energy installations in Portugal. He told *Nature* "the bank wanted publicity, but the house was still not finished at the time of the inauguration; so we connected a plastic hose to the plates to prove to the press that they heated the water." Falconer's firm has been importing MIROMIT plates — selective coating plates developed by the Tabor group — from Israel since 1961.

The other solar energy pioneer is Mr Neves da Silva, who obtained a Portuguese patent for a simple painted flat solar collector in the early 1960s. He is director of PRETEC, a mechanical construction firm, and he first attempted to build his collectors from national materials. "We tried to use cork, Portugal's main export product, as an insulator, but the price was too high; so we have to use glass wool like everyone else."

Both men are enthusiastic practical engineers. They have believed in solar energy for the past twenty years in spite of general indifference and incredulity. "In the sixties I had to deal with an engineer who stayed up all night hoping to catch me heating up the water in the reserve tank with an electric resistor. That was the only way he could imagine I could get hot water during the day from my system" recalls Mr Falconer.

"In the early days solar energy was no more than a toy for the very rich. Today it may still be mainly 'to show to the English', as the Portuguese saying goes." Indeed, a stone's throw from the casino in the resort town of Estoril on the fashionable Costa do Sol stands a huge mansion which is, according to its owner, the only solar equipped house and swimming pool in Portugal. Among the bougainvillea trees and parterres of geraniums are interspersed rows of solar collectors; the mansion itself displays several striking black overhangs on the south side. "My pool is heated up by 24 panels; my family has used it 90 days per year since 1961" says Virgilio Gomes Borges, one of the brothers of the Borges Brothers Bank. "I use 32 panels in the sanitary water circuit; additional heat storage is provided by the electrical company's reduced night rates."

Using the Sun's light remains an elitist activity haphazardly conducted in the midst of abundant talk about energy crisis on the one hand and Portugal's exceptionally favourable climatic situation on the other. Tourism officials claim 244 sunny days per year. A more objective but no less encouraging way of rating Portugal for its potential is through its atmospheric transmission ratio — of the energy received on a horizontal surface on the Earth to the energy received on an equal surface at the top of the atmosphere, averaged over a year. According to Dr Collares Pereira, the only Portuguese physicist with a PhD in solar energy work, that ratio varies between 0.3 for high latitude rainy climates and 0.7 for clear desert locations like Albuquerque in New Mexico. Lisbon's ratio is 0.65 (and the south of Portugal is considerably sunnier) while New York and New Jersey, at the same latitude, hardly reach 0.5.

The local capacity for building solar plates has improved over the years. Mr Neves da Silva remarks that back in 1962 he operated as "an artisan", but today PRETEC would be capable of producing 100 collectors per year. What is missing, however, are serious orders and some planning and stimulation on the part of the government. At the opening session of a Franco-Portuguese seminar on solar energy last March, Mr Sidonio Pais, General Director for Energy of the Ministry of Industry, declared that it was impossible for him to be specific about solar energy developments at the moment because "our government does not have yet an energy policy".

At the same seminar, the Secretary of State for Higher Education praised the ecological advantages of solar energy but spelled out no plans for the future.

In the private sector several religious schools have put up collectors to heat up sanitary water. "It is quite well adapted to the kind of financing that this type of institution can do" says Neves da Silva who built the systems, "religious groups can easily raise money once-off for such a project, and solar heating does not give rise to monthly costs afterwards."

For the future the Ministry of Health has in its files a construction program for 27 health centres throughout the country, each equipped with 75 solar panels for sanitary water heating. It should come up for bidding soon.

Maurice Bazin

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'Those who attack nuclear energy show a cynical denial of human ingenuity'

THREE Mile Island confirmed what many of us in the nuclear enterprise had suspected — that the "China Syndrome", rather then proliferation or even waste disposal, would ultimately decide the public's acceptance of nuclear energy. Nor is this unreasonable. Proliferation is an issue far from most people's perception; more than that, it faults nuclear power by association, not by intrinsic connection. As for waste disposal, despite the violent arguments, the nuclear community could always point to plausible technical solutions for wastes (one must remember that 800 years after wastes are created, their toxicity has fallen below that of the original uranium from which the wastes were created). But for the 15 billion curies contained in a 1000 megawatt reactor, we could never say that the chance of a serious accidental release was zero. Three Mile Island carried this message directly to the public; the public must decide whether it can accept this risk. Chancellor Schmidt's recent call for an international review of safety as a sequel to the International Nuclear Fuel Cycle Evaluation study of proliferation resistance is therefore most appropriate.

The fact that deaths caused by nuclear energy, are on average, considerably fewer than those estimated for most energy sources (including many solar sources if they are backed up by non-solar systems), seems to me rather beside the point. Risk as perceived by the public is not simply the product of probability and consequences — the first moment of the probability distribution of consequences. The very high moments of the distribution — very low probability high consequence events that contaminate large stretches of land — are probably more important. Thus a most important technical fix for nuclear energy would be a means of minimising the amount of land that conceivably could be contaminated in the worst possible accident. Siting reactors underground could achieve this, though at considerable expense. The recently published report on ungerground siting to the California Energy Commission concludes that comparable improvements can be achieved at much lower cost with more elaborate containment systems.

Three Mile Island suggests that even an accident that imposed an average additional radiation burden 1% of the natural background on those within 20 miles is unacceptable. For nuclear energy to survive, we must reduce the probability of *any* serious malfunction much below the 1 in 20,000 per reactor-year estimated in the Rasmussen report, as well as reducing any possible consequences.

Can we fix nuclear energy? I believe we can, but it will require important institutional changes as well as better technology. I am therefore most enthusiastic about the plan announced earlier this year by the Tennessee Valley Authority, the largest electrical generating entity in the United States, in response to Three Mile Island. The main elements of the TVA plan are:

- the establishment of a separate operating entity with the Office of Power that is responsible only for nuclear generation;
- the establishment of an independent Nuclear Safety Review Board that reports directly to the Board of Directors of TVA;

Alvin Weinberg (right) explains why, despite the Three Mile Island accident, he is still a believer in nuclear energy

- the professionalisation of the cadre operating nuclear reactors — operators will receive training equivalent to a bachelor's degree, and salaries high enough to attract and keep the best, even though this places them at an advantage as compared to operators of fossil fuel or hydroelectric plants;
- more extensive plans for evacuation, and for keeping the public informed on the status of its nuclear plants.

In addition to these institutional changes, TVA is, on its own, proposing a number of technical improvements for its reactors that go beyond what the Nuclear Regulatory Commission requires. TVA, being the largest American utility, is well equipped to analyse and then implement safety mesures that a smaller utility might not be in position either to recognise or to pay for. It is to TVA's credit that one of its safety engineers, C. Michelson, had visualised essentially the Three Mile Island sequence a year before it actually happened, and had called the attention of Babcock and Wilcox, (the nuclear steam suppliers) and the Nuclear Regulatory Commission to the deficiency.

Although not mentioned in their plan, I would hope that TVA will confine future reactors to its existing seven sites rather than allowing nuclear sites to proliferate. The sites would be expected eventually to develop into powerful nuclear centres. They would be invested with a commitment of permanence: spent fuel elements, low level wastes, and decommissioned reactors could be stored for a long time on them. After 100 years the high level wastes would be producing little heat and the low level wastes and reactors, though not innocuous, would be much easier to handle.

The TVA plan, together with confinement of the nuclear enterprise essentially to existing sites, seems to me to constitute an acceptable nuclear energy system. TVA, being large, self-contained, and able to set its own rates, is better able to implement such a policy than are most of the private utilities. But I believe the private utilities ought to move strongly in this direction — by establishing *nuclear* generating consortia, perhaps like the Yankee Atomic Electric Company, in each of the nine regional Reliability Councils; or possibly by assigning generation of all nuclear energy in a region to a single utility that has the strength to take on this responsibility. Such a nuclear generating company, from the president down to the operators, would think, act, and know the nuclear business, with all its responsibilities and hazards.

Will an aggressive acceptance by the US, or even the whole world, of some version of the TVA plan plus confined siting and technical fixes, be enough to tide nuclear energy over the present disaffection? I certainly hope so. The TVA plan, with its standby mobilisation of experts to respond to any contingency, ought to give fullest opportunity for the exercise of human ingenuity. The serious nuclear accidents, though they were precipitated by human error, were contained by human ingenuity. At Three Mile Island, shutting off the high pressure injection pumps was perhaps the key operator error; but only 12 curies of ^{113}I were released — some 2000 times less than was released at Windscale in 1958. A key manoeuvre in preventing more iodine from escaping was the replacement of the iodine filters a few days after the incident began. This was carried out faultlessly by experts from various nuclear installations.

I consider myself to be a not uncritical advocate of nuclear energy: an advocate because I believe we need nuclear energy and the alternatives to it are faulted; not uncritical because I have been close to the technology for enough years to recognise its deficiencies. Despite Three Mile Island, I remain an advocate. Those who wish to extirpate nuclear energy base their position on an unquestioning acceptance of human fallibility and a cynical denial of human ingenuity. The two major accidents in the United States, Browns Ferry and Three Mile Island, suggest to me that this is an unjustifiably pessimistic assessment of the human condition. Rather, as the TVA Plan reveals, there is much room for creating institutions that will encourage ingenuity, and on which we can base an acceptable nuclear energy. □

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correspondence

'Beast and Man'

Sir, — I have only just seen Professor Stuart Sutherland's very interesting review of my book *Beast and Man* (28 June page 83.) May I say something in reply to the difficulties he raises?

He says that the main aim of my book "seems to be to establish a secure basis *for ethics*" and that, like "most previous attempts to establish a secure basis *for an ethical system*", it fails. (The italics are mine). Two projects might be meant, of which the first is certainly not mine —

- A "secure basis for an ethical system" would be a justification of one particular set of moral views against others. This would itself be a piece of ethical reasoning.
- A "basis for ethics" by contrast seems to mean something much more general — a justification or explanation, from outside, of the whole range of thought which can be called ethical or moral. Confused people often run these two enterprises together, supposing that the second will do the work of the first. I think this is what Professor Sutherland objects to, and I share his objection. There can be no moral justification of morality as a whole any more than there can be a mathematical justification of mathematics as a whole; neither from outside nor from inside these areas of thought would such an enterprise make sense, nor is it needed. In the case of morality, the notion that it is needed, and is lacking, stems largely from Nietzsche's ambiguous talk of 'immoralism'. Nietzsche often spoke of this as a comprehensive attack on the very possibility of morality. But in fact his campaign was an intelligently limited one, directed against certain particular human weaknesses and particularly against contemporary bourgeois notions. It was conducted in entirely moral terms, powered by fierce and unmistakeable moral indignation, and successful so far as his readers approved of it. It was a piece of ethics. The wider program of attacking or justifying morality as such remains obscure.

It was therefore no purpose of my book to provide defence agains this hypothetical general attack. I am interested, as most serious moral philosophers have been, in explanation rather than in instant justification, in understanding rather than in proof. For ethics as for any other area of thought, we need to *understand* our basic concepts, to resolve conflicts among them, and to relate them systematically to those used in relevant neighbouring fields. That is the sense in which people speak of the 'foundations' of mathematics. Nobody supposes these to be a set of non-mathematical arguments, guaranteeing mathematical truths from outside. In the case of ethics, however, people do for some reason tend to expect this, and even when they give their attention to the concepts, have a remarkable tendency to insist that they shall be simple. Hume's question, 'whether the foundation of morals lies in reason or in feeling?' has been only one of a whole series of grossly over-simple dilemmas, which it is the point of my book to defuse. I draw attention to the complexity of the relevant facts, to the unreality of various simple rulings, and particularly of those, fashionable in the first half of this century, which isolated moral thinking from the natural facts. During this epoch, a variety of reasons were given for tabooing many useful, indeed essential, concepts such as 'natural' which refer to facts, but are necessary to explain our value judgments. I do not think I can now trespass on your space with a full answer to Professor Sutherland's worries about my treatment of these. But perhaps it will help if I emphasize that I do regard them as explanatory, and not as an underhand short cut to justifying local prejudice.

Yours faithfully,

Mary Midgley

University of Newcastle upon Tyne, UK

East Europe: valuable information

Sir, The need by science of 'extensive, systematic and friendly international ties' noted by your corresondent, Lee Lorch, (13 September, p 98) is one which I am confident is recognized by *Nature* and by the overwhelming majority of its readers. Having worked at various times in research laboratories in half a dozen countries, including the USSR, I have personally had the good fortune to benefit directly from existing ties and have, I hope, made a modest contribution to furthering them.

Lee Lorch accuses *Nature* of allowing itself to be used by propagandists "anxious to whip up an hysterical ill-will against East European science", an accusation so far-fetched that it would not merit refutation if it were not coupled with a scornful and vicious personal attack on your regular contributor, Vera Rich.

Her articles are a valuable source of information about science and technology in Comecon countries. Of course it is incomplete, of course it is biased; Ms Rich writes rather more often on environmental matters than on high energy physics, and as far as I know has never written about Polish research in general relativity. But omission and bias are not the same thing as prejudice, still less contempt and propaganda.

To be sure there have been occasions, sad to say not infrequent, when she has reported on the action of the authorities against individual scientists, their dismissal from employment, arrest, or worse. The cases which she has reported have been in the USSR and in East Europe — but that is the region of her specialist interest and knowledge and others in your journal have balanced the record of injustice with reports from elsewhere.

The achievements of Soviet science are beyond question; the distinction of her scientists and engineers and their contributions to the advancement of knowledge command the admiration and respect of the world community of science. The mutual benefits that flow from international cooperation, the free exchange of ideas and the unrestricted travel of scientists themselves are widely recognized. Your journal indeed has a responsibility, the greater because of its eminence, to report on developments in science in East Europe as elsewhere. But it has a responsibility too to report with the candour and good faith of a true friend of international cooperation the less favourable aspect of the policies of those countries insofar as they affect science and scientists. To pretend otherwise is no way to encourage the ties of friendship: it is to acquiesce in injustice.

Yours faithfully,

John M Charap

Queen Mary College, University of London

'MHC' nomenclature

Sir, — Rolf Zinkernagel is too modest (14 June, page 618). The revolution which he and David Katz started has changed our thinking about the 'MHC' (major histocompatibility complex) to an extent which makes nonsense of the conventional nomenclature used in the otherwise excellent book which he reviews. His thesis is now generally accepted that MHC-coded molecules (other than complement components) evolved as signals for T cell subsets, which enable the immune system to make an appropriate choice between these subsets when combatting varied forms of infection. If one takes this point of view, present nomenclature is quite misleading. The very term, 'major histocompatibility complex' place the emphasis on transplantation, not infection. It perpetuates the historical accident which led to its discovery, but misleads as to its normal function.

Terms such as cytotoxicity-defined or lymphocyte-defined are open to the same criticism. The term 'I region' with the associated terms Ia-molecules, I-A, etc. implies falsely that H-2K and D molecules are not immunoregulatory. In fact, they are so, and in a way which appears exactly to parallel the immunoregulatory functions of Ia-molecules, except that they control cytotoxic T cells rather than helper T cells. Furthermore, gene substitutions at K and D regulate immune responsiveness of cytotoxic T cells just as substitutions in the I region regulate the responsiveness of helper cells. The whole complex, again with the exception of the complement components, seems to be equally immunoregulatory. Indeed, one really ludicrous usage is creeping in: to refer to the I-region genes as *the* IR genes. No doubt, many if not most gene substitutions perturb the immune system, just as most known substitutions perturb the nervous system: it's just that behavioural effects are more obvious.

We hesitate to suggest alterations, chiefly because it annoys others so much when one does so. T cell-activating complex? Cytotoxic cell activating molecules versus helper cell activating molecules? There are plenty of problems still: the aberrant presence of Ia-molecules in glandular epithelia and T cell products, the odd behaviour of I-J molecules, questions about restriction elements on B cells, and so on. What is certain is that so long as the present nomenclature is used we shall have to go on explaining to newcomers that the only way to understand the MHC - and hence what the majority of cellular immunologists these days are up to -is to forget about what the terms seem to mean.

Yours faithfully,

J. Michael Cecka, Alexander Kamb,
Ursula Kees, N. Avrion Mitchison

University College, London, UK.

Radio and the 'experts'

Sir, "A pity" — you say (23 August, page 619) — that more qualified people fail to participate in phone-in and open-line radio programmes.

The reason is simple: most of us are at work, so don't hear the programme anyway; those not at work presumably would not wish to advertise the fact!

Yours faithfully

I.P.C. Gaut

Optrex Ltd, Hampshire, UK.

news and views

Vanadate — a new tool for biologists

from T. J. B. Simons

RESEARCH on the biological actions of vanadate has increased enormously during the past three years. Vanadium compounds can be highly toxic to man and animals and yet removal of the minute quantities normally present in the diet leads to deficiency disorders in experimental animals[1]. The biological basis for the apparent requirement for vanadium is still unknown but currently its effects on certain enzymes — notably the ATPases — are being studied intensively.

The stimulus to the present interest in vanadium came when vanadate was identified[2,3] as the impurity in ATP derived from equine muscle, which inhibited dynein ATPase[4] and the membrane (Na + K)-ATPase[5,7]. The impurity was present at only 1/3,000 the molar concentration of ATP.

Since then studies on both the physiological and the biochemical effects of vanadate have revealed a wide range of activity. Particularly interesting is its apparent selective inhibition on some ATPases, which has led to its use as a selective probe. It also inhibits several other enzymes (acid phosphatase[8], alkaline phosphatase[9], phosphofructokinase[10] and adenylate kinase[11]; on the other hand it stimulates adenyl cyclase[12]. It can replace phosphate as a substrate for glyceraldehyde 3-phosphate-dehydrogenase. This leads to the formation of an unstable analogue of 1,3-diphosphoglycerate, similar to that produced by arsenate[11]. Its physiological effects include diuresis, natriuresis[13], increasing the force of contraction of the heart[14] and vasoconstriction[15].

Two forms of vanadium in particular have been studied and have been found to have different effects. The first, vanadate, exists mainly as $H_2VO_4^-$ anions in dilute solutions at neutral *p*H, but it polymerises at concentrations above 10^{-4} M (ref. 16). Monomeric vanadate is structurally analogous to phosphate and this could account for its inhibitory effects on enzymes concerned in phosphate transfer or release reactions. Like phosphate, vanadate enters human red blood cells by the anion transport mechanism[17]. Inside cells however, vanadate often seems to be reduced to vanadyl ions (VO^{2+}) which bind to proteins[17-19]. Vanadyl ions have different effects from vanadate: they are powerful inhibitors of alkaline phosphatase, but seem not to affect the (Na + K)-ATPase[18,20,21]. One complicating factor in experiments is that vanadyl solutions are readily oxidised to vanadate by the atmosphere.

Inhibition of (Na + K)-ATPase

Cantley and Josephson have done much to characterise the inhibition of (Na + K)-ATPase by vanadate (ref. 22 and see refs therein). (Na + K)-ATPase is embedded in cell membranes and couples ion movements — Na efflux and K influx — to the hydrolysis of ATP. This is brought about through a phosphoenzyme intermediate, whose formation and breakdown are promoted by Na and K ions, respectively. The enzyme is dimeric, and shows both high and low affinities for ATP-binding and ATP hydrolysis[23]. The two halves are thought to be coupled in an anti-parallel fashion, with phosphorylation occurring on one subunit at about the same time as dephosphorylation occurs on the other[24]. Vanadate is a reversible inhibitor. It binds most strongly in the presence of high concentrations of Mg and K ions, when 40 nM vanadate gives 50% inhibition[2]. Detailed binding and competition studies show that it binds to two sites — a high and a low affinity site, and that vanadate at its high-affinity site competes with ATP at its low-affinity site[22]. Phosphate is a poor product inhibitor, and competes for the high-affinity vanadate site. This raises the possibility that the phosphate release site may be the same as the low-affinity ATP-binding site. It could imply that the ATP-binding sites go through a cycle of high and low affinities towards ATP, corresponding to phosphorylation and dephosphorylation, respectively. Supporting evidence is tenuous as yet: micromolar concentrations of vanadate only inhibit ion transport from the inner surface of the membrane[17,26], where ATP acts.

Another interesting point concerns the promotion of vanadate binding by external K ions. This requires much higher concentrations of K than are needed to stimulate the (Na + K)-ATPase, suggesting that in this case K acts at sites different from those which normally activate the enzyme[26-28]. When K is absent, ATP is still hydrolysed and Na efflux continues, but at slower rates. The enzyme now shows only high affinity sites for ATP[23], and vanadate inhibits the ATPase with relatively low affinity[22]. Internal vanadate has no effect on the Na efflux from squid axons in K-free sea water[26], in agreement with this observation.

Inhibition of dynein and other ATPases

Dynein is an energy-transducing protein responsible for the mobility of cilia and flagella. It can be extracted from them and shows a Mg-ATPase activity that is 50% inhibited by 0.1 to 1 μM vanadate[29,30]. Early experiments showed that vanadate does not inhibit myosin or actomyosin ATPases, except at much higher concentrations[29,31,32] and so can be used as a selective inhibitor to investigate cell motility. A difficulty with this kind of investigation is that the cell membrane is relatively impermeable to vanadate. For example, the flagella of sea urchin sperm are unaffected by up to 10 μM vanadate, but their beating is stopped by as little as 4 μM after the plasma membrane is treated with detergent[30]. Perhaps the most interesting observation has been made by Cande and Wolniak[32], who found that 10 to 100 μM vanadate inhibits chromosome movement and spindle elongation at anaphase in lysed mamalian tissue culture cells, which suggests a role for dynein in the spindle mechanism. More recently, Goodno has found that vanadate really is a powerful inhibitor of myosin, forming a ternary complex with ADP, but the onset of inhibition is so slow that it had previously been overlooked[33].

The sarcoplasmic reticulum (SR), and many cell membranes, have a Ca-ATPase, which couples Ca ion transport (out of cells or into the SR) to ATP hydrolysis. The SR enzyme has many structural similarities to (Na + K)-ATPase[34], so it is rather surprising that low concentrations of vanadate have no effect on it[31], although they do inhibit the red cell Ca-ATPase[35]. This point needs further investigation. The active transport of Ca out of squid axons seems to be partly dependent on external

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T.J.B Simons is in the Department of Physiology, King's College, London.

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Na, and partly on internal ATP. Vanadate selectively inhibits ATP-dependent Ca extrusion[36], which suggests that it is brought about by a Ca-ATPase similar to that in the red cell membrane. Selective inhibition by vanadate might therefore be used to investigate the mechanism of Ca extrusion from heart, smooth muscle and other cells.

Physiological effects

There is an extensive literature on the toxic effects of vanadium salts, and some current observations, of effects on the heart or circulation for example, are not really new[37]. Recent work has shown that it exerts a positive inotropic effect (increases the force of contraction) in ventricular muscle, but a negative inotropic effect on cat and guinea-pig atria[39, 40]. Since the (Na + K)-ATPase in membrane preparations from both ventricles and atria is inhibited by vanadate, it must act on cell components other than the (Na + K)-ATPase. Action potential duration is lengthened by vanadate in guinea-pig papillary muscle (ventricle), yet shortened in atria[39]. This correlates well with the inotropic effect. Catecholamines have a positive inotropic action on the heart, lengthening the action potential, and augmenting inward Ca current. This is associated with a stimulation of adenyl cyclase and a rise in intracellular cyclic AMP[41]. Vanadate is known to stimulate adenyl cyclase[12, 40], so this could account for its positive inotropic actions, but not the negative effect in the atria. Obviously, much work remains to be done to understand the actions of vanadate on the heart.

Recently, deSousa and Grosso reported that vanadate blocks the cyclic AMP-induced stimulation of Na and water transport in amphibian epithelia[42]. The first finding, that Na transport is inhibited, can easily be explained by inhibition of (Na + K)-ATPase. The block of vasopressin- or isoprenaline-stimulated passive water permeability is much more interesting. Vasopressin increases the water permeability of many epithelia. This is associated with a rise in intracellular cyclic AMP, and can be mimicked by exogenous cyclic AMP. Cyclic AMP is thought to affect the phosphorylation of one or more membrane proteins that control permeability[43]. The effect of vasopressin can be blocked by colchicine, a microtubule-disrupting agent. This observation has led to the suggestion that microtubules are involved in the action of vasopressin[43]. Dynein is associated with microtubules, and can be inhibited by vanadate. Perhaps it also plays a part in the vasopressin response[42].

A regulatory role for vanadate?

Several authors have suggested that vanadate may have a regulatory role in mammalian tissues[2, 18, 22, 30]. Small concentrations of vanadium are present, and these would be sufficient to affect the (Na + K)-ATPase, if it were present as vanadate. Regulation could be provided by controlling the vanadyl/vanadate redox reaction. However, all that can be said at present is that there is no evidence for or against this hypothesis. □

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The genesis of a void lattice

from Robert W. Cahn

WHEN a metal crystal is irradiated with neutrons or other more massive projectiles then, within certain temperature limits, it acquires a population of microcavities known as 'voids': these arise as a result of the preferential agglomeration of the crystal vacancies which are formed together with interstitial atoms as a result of irradiation. In some metals, voids have been found to arrange themselves in an orderly three-dimensional array – a 'void lattice'. Properly speaking, this is a superlattice, parallel to but coarser than the underlying crystal lattice. The classic instance is the void lattice in irradiated molybdenum, which mimics the body-centred crystal structure of molybdenum itself: but whereas the interatomic distance in the molybdenum lattice is a fraction of a nanometre, neighbouring voids in the void lattice are separated by a distance of 18-27 nm, according to the temperature at which it is created.

A very extensive and effective research effort has developed during the past decade into the genesis and growth of individual voids in the alloys used to contain nuclear fuels and also into the inhibition of void formation; but the origin of the void 'lattice', which is of purely fundamental interest, has only intermittently attracted attention. This is a pity, because the phenomenon is intriguing.

A short paper has just been published in which the known facts concerning void lattice formation in molybdenum are assembled and complemented by some further measurements (Liou, Smith, Wilkes & Kulcinski *J. Nucl. Mater.* **83,** 335; 1979). Molybdenum is the metal in which the existence of a void lattice was originally discovered by Evans in 1971. The new feature which Liou and his collaborators have now established is that voids are originally formed (within a temperature range of about 800-1100 °C) always in a random pattern, and a void lattice is formed only later when a much higher radiation dose has been absorbed and individual voids have grown to a sufficient size. Any theory of the formation of the void lattice therefore has to be based upon the progressive ordering of an initially random dispersion of voids, and indeed such a theory must come to grips with the question of why voids have to grow to a certain size before a lattice can form. Notions such as the nucleation of voids on an ordered pattern of dissolved gas atoms or on an ordered array of dislocation loops (such an array has been observed) are not acceptable.

The stability of a void lattice has been satisfactorily explained by Harwell theorists (for example, Stoneham *J. Phys. F* **1,** 778, 1971). Spherical voids would attract each other at all separations in an elastically isotropic medium, and it is only in an anisotropic medium that a pair of voids can rest stably at a particular separation. Stoneham has proved that a b.c.c. or f.c.c. lattice of voids is stable in molybdenum, though the predicted ratio

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Robert Cahn is Professor of Materials Science in the University of Sussex.

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of the separation of voids (in the observed b.c.c. void lattice) to the void diameter is smaller than the values actually observed. Stoneham's theory explains why such a lattice is stable once formed, but not how it is established in the first place.

Liou and his collaborators point out that the formation of a void lattice is analogous to the alignment of precipitate plates on different planes to form a preferred, stable array. This is very commonly observed in alloys formed by spinodal decomposition or by ordering, but had not hitherto been properly analysed. The theoretical basis of this phenomenon has now been rigorously treated by Perovic, Purdy and Brown (*Acta Met.* **27**, 1075; 1979), who calculate the total elastic energy of a regular array of plate-shaped precipitates in a matrix, the precipitates lying on three mutually orthogonal planes. They show that such an array, subject to certain conditions (primarily a low interfacial energy and a large, elastically accommodated misfit between matrix and precipitate) can stabilise at a particular value of the separation of adjacent precipitates, so that further coarsening of the array is inhibited. This presumably is what happens in the formation of a void lattice, though in fact it is not yet known whether a fine void lattice forms first and then coarsens until it becomes stable, or whether the initially disordered voids move about in a kind of Brownian motion until the relative positions of a small group of voids happen to be correct to nucleate a lattice at its proper stable lattice parameter. In the case of a precipitate array, each precipitate plate creates an anisotropic strain field around itself, and an isotropic medium is assumed, for simplicity; whereas in the case of voids, it is only the anisotropy of the medium which allows a void lattice to form at all.

Electron microscopy has shown that an array of precipitates can coarsen progressively by simultaneous dissolution of some portions of a precipitate plate and growth of other portions (Saunderson, Wilkes & Lorimer *Acta Met.* **26**, 1357; 1978), and voids or gas bubbles are known to migrate and merge by a similar mechanism. The growth of voids can be inhibited by small amounts of impurity, which may be fission products such as technetium; the impurity can gather at the void wall and prevent plating out of further vacancies into the void. Because of this, voids may even shrink in the later stages of irradiation (Evans *J. Nucl. Mater.* in the press). In such instances, the eventual shrinkage of the void population is in competition with the formation of a void lattice (which requires a minimum size of void to be attained). What is really required now is simultaneous ion-bombardment and electonmicroscopic observation of the same samples so that the stages of formation of a void lattice can be observed while they actually happen.

Most published photographs of void lattices show them to be distinctly defective (for instance, the photograph on page 338 of Liou's recent paper); the periodicity has a good deal of scatter. It would be interesting to know whether a void lattice, once formed, gradually loses such regularity as it possesses if it is progressively heated; just as a conventional superlattice in, say, Cu_3Au gradually disorders as the alloy is heated. No experiments on annealing of void lattices seem to have been attempted, nor has anyone attempted to improve the regularity of a void lattice by very slow cooling (which is the method used to obtain highly perfect conventional superlattices). There are plainly a number of parallels between void lattices and conventional superlattices which deserve critical examination. □

A spherical structure for allophane

from Ian Smalley

Koji Wada of Kyushu University has produced and collected evidence which strongly suggests that the clay mineral allophane, long regarded as an 'amorphous' constituent of soils, has a unique spherical structure. Although plates and tubes have already been encountered in clay mineralogy, this seems to be the only occurrence of spheres (*Proc. Int. Clay Conf.*, eds Mortland & Farmer, 537; Elsevier, New York, 1978). Taylor and Wilson (*Clays and Clay Miner.* **27**, 261; 1979) describe allophane as consisting of an aluminosilicate core, about 5 nm in diameter, coated to a variable extent with amorphous aluminium hydroxide. The clay surface has hydroxyl and hydronium groups associated with the polymeric hydroxide and the aluminosilicate, and these (by their protonation and deprotonation) are responsible for the *p*H-dependent particle charge developed in aqueous systems. (The nature and properties of variable-charge soils containing allophane will be discussed at the International Society of Soil Science meeting in New Zealand in 1981.)

Allophane usually coexists with imogolite, but the two can be distinguished easily by their shapes, spherical allophane contrasting with tubular imogolite. In 1971 Kitagawa first discovered by high resolution electron microscopy that allophane consists of spherical, hollow unit particles with an average diameter of 5.5 nm. Hemni and Wada (*Am. Miner.* **61**, 379; 1976) observed that similar particles occurred in sixteen allophanic clays, irrespective of their ages and lithological compositions, or the origins of the volcanic ashes and pumices from which the clays were separated. They estimated the external diameter of the particles to range from 3.5 to 5.0 nm and the thickness of the wall to be 1.0 nm or less. The thickness of the wall is broadly consistent with that of a defect kaolin structure.

Wada and Wada (*Clay Miner.* **12**, 289; 1977) determined the density of allophane by float-sink tests in CH_3OH-, CH_3COCH_3-, C_6H_6- and CCl_4-$C_2H_2Br_4$ mixtures as 1.84, 1.94-1.98, 2.35 and 2.35-2.39 g cm^{-3} respectively. Either water molecules only fill the voids of the spherule wall, or all liquid molecules (including $C_2H_2Br_4$) occupy these spaces, the first possibility applying if the wall contains small pores and the second if it contains large pores. It is reasonable to assume that 'defects' in the silicate structure produce such pores or openings in the wall, and the passage of water molecules through these has been confirmed by the ease with which OH ions can be replaced by their deuterium-containing equivalent (OD^-) in allophane.

Wada concludes that the structural formulae for allophanes are in general consistent with the available electron optical and density evidence. The modification of the kaolin layer caused by the size and shape of the unit particles, and by the occurrence of defects, may account for several features of allophanes, especially the absence of both basal and two-dimensional X-ray reflections, the broadness and poor resolution of Si-O-(Al) absorption bands in the 1100-900 cm^{-1} region of the infrared spectra, and the remarkably continuous loss of water with increasing temperature due to dehydration and dehydroxylation. The observed electric charge and acid characteristics of allophanes are also broadly consistent with what is predicted from the structural formule. □

Ian Smalley is with the Soil Bureau, Department of Scientific and Industrial Research, Lower Hutt, New Zealand.

Soil biology and land use

from Clive A. Edwards

A recent symposium* addressed the often neglected subject of the impact of human activities on the soil — the resource on which all plant and animal life depends. Although man's use of the land can often have damaging effects on the soil, work presented at the symposium showed that with care, such effects can be minimised, and careful manipulation of some of the multitide of soil organisms can benefit man by increasing soil fertility, and can help to reclaim polluted land and dispose of an increasing volume of human and animal waste.

There has been much discussion on how

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pesticides, used in agriculture and in aerial spraying programmes, affect soil ecosystems and a day was devoted to assessing how pesticides affect soil communities and in particular their effects on earthworm and arthropod populations. Work at Rothamsted Experimental Station in England has shown that relatively few pesticides are toxic to earthworms; those that do kill these animals which are important in maintaining soil fertility, include the insecticides chlordane, phorate, parathion and carbofuran, the nematicides D-D, methyl bromide, metham sodium and chloropicrin, and the carbendazim-based fungicides. Work by A.B. Broadbent and A.D. Tomlin, (Canada Agriculture, London, Ontario) and others has shown that pesticides do not have a drastic overall effect on soil arthropod communities but that rather they tend to upset the precarious balance in soil ecosystems, sometimes leading to spectacular upsurges in numbers of those organisms unaffected by a pesticide when their predators were killed; and even, in some instances, new pest problems.

Keen interest was attracted by the work of Roy Hartenstein and his colleagues (College of Environmental Science and Forestry, Syracuse) on the use of earthworms to process human sewage. As restrictions on the dumping of sewage at sea are implemented so waste disposal problems are becoming more serious. The Syracuse workers have found that certain species of earthworms, particularly *Eisenia foetida,* can break down raw sewage into a soil-like material, rich in nutrients and suitable for addition to agricultural land. This process takes about 2 weeks and the commercial development of such a process on a large-scale basis is being investigated and seems likely to succeed. The earthworms produced may be useful as a protein supplement in animal feed. One obstacle to such a process is the rather large concentrations of heavy metals such as lead and cadmium that are often present in sewage, and which would be undesirable in agricultural land from which they might move into edible crops. One group of workers reported investigations into the uptake of heavy metals into earthworm tissues and methods of neutralising the effects of heavy metals on and uptake into crops grown on sewage-amended soil (C. Andersen, Royal Veterinary and Agricultural University, Copenhagen, Denmark).

A third subject that attracted interest was the effects of different kinds of animal wastes added to agricultural land on soil organisms. Workers at University College, Dublin (J.P. Curry & D.C.F. Cotton) have shown that the initial effects of slurries was to decrease earthworm populations in soil to which such wastes had been added drastically but within 7 months populations had recovered and greatly exceeded those in untreated soil. The overall conclusion was that the addition of animal waste to soil tends to favour populations of many soil invertebrates, particularly earthworms, so there seem to be few ecological objections to such a practice.

Surface mining is becoming even more widespread and there is keen interest in the reclamation of such land. The effects of mining iron ore, coal and bauxite on populations of soil organisms were discussed. The general impression was that most mining programmes tend to decrease both species diversity and populations of many soil organisms.

The possibilities of restoring these and other highly disturbed sites to normal biological activity are being investigated by D. Parkinson *et al.* (University of Calgary, Alberta, Canada) by adding different types of surface amendments. They found sewage sludge to be the most effective amendment in terms of plant production and microbial development.

It seems clear from the topics discussed at the symposium that the potential for profitable manipulation of soil organisms to minimise the adverse effects of human activities on soil is considerable and merits much further attention. □

Clive A. Edwards is in the Entomology Department at Rothamsted Experimental Station, Harpenden, Herts.

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*The VII International Congress of Soil Biology was held on 30 July-4 August at the State University of New York, Syracuse. The proceedings will be published with financial assistance from the US Environmental Protection Agency.

Neurotoxins in Nice

from R. C. Hider

MANY of the most potent animal toxins known are specifically directed against critical membrane proteins of the neuromuscular system. As a result of their specificity and high affinities, biochemists and physiologists have been using many such molecules to great advantage over the past decade. This point was clearly demonstrated at the recent successful EMBO workshop on neurotoxins held in Nice*, where a largely European audience heard presentations on the acetylcholine receptor, the sodium channel and presynaptic membranes.

Postsynaptic toxins

Cobra venom possesses extremely powerful toxins which cause the paralysis of vertebrate skeletal muscle. These small proteins (molecular weight 6,000-7,000) achieve this by binding tightly to the acetylcholine receptor (AChR) which is concentrated at neuromuscular junctions. A toxin-bound receptor is no longer responsive to the natural agonist acetylcholine. The detailed structure of the acetylcholine receptor has been a controversial subject for some years. At the meeting J.P. Changeux (Pasteur Institute) presented compelling evidence that AChR is a hexamer of six identical subunits of molecular weight 40,000. Preparations of this protein have have been successfully incorporated into vesicles and judging from the rapid binding kinetics of a fluorescent agonist, the reconstituted AChR is in a similar state to that found in native membranes. Agonists increase the permeability of these vesicles towards Na ions, the increase being specifically inhibited by α-bungarotoxin (a snake neurotoxin) (Changeux, Heidmann, Popot & Sobel *FEBS Lett.* 105, 181; 1979). A similar mechanism has been established for native AChR *in situ* by electrophysiological experiments. A problem with reconstitution experiments of this type, as pointed out by Changeux, is that the presence of trace quantities of other proteins could be associated with the ion permeability changes. A report from M. Raftery (California Institute of Technology, Pasadena) could have a real bearing on this point because he proposed, on the basis of a large body of evidence, that the receptor is composed of four different proteins (Vandlen, Wu, Eisenach & Raftery, *Biochemistry,* **18,** 1845; 1979). Furthermore he demonstrated that snake neurotoxins bind to two of these components (Raftery *et al. Adv. Cytopharmacol.* **3,** 27; 1979). Both Changeux and Raftery use postsynaptic membranes of the electric organ of *Torpedo* in which AchR constitutes up to 40% of the protein. Significantly, Barnard when isolating AChR from mammalian muscle also finds a single protein of approximately 40,000 molecular weight (Shorr, Dolly & Barnard *Nature* **274,** 283; 1978). Barnard also considers that the ligand recognition unit is possibly an oligomer of six identical units.

If this relatively simple concept proves to be correct then AChR will soon be subjected to classical enzymological studies. Indeed, Changeux presented the N-terminal sequence of the 40K protein (Devillers-Thiery *et al. FEBS Lett.* **104,** 99; 1979) and many advances have been made on the interaction between the snake neurotoxins and this receptor (Maelicke *et al. J. biol. Chem.* **252,** 4811; 1977). The X-ray structure of two such toxins are now established, erabutoxin-b (Kimball *et al.*

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*EMBO workshop on the Structure and Function of Neurotoxins (24-26 July, 1979) organised by Michelle Lazdunski, Centre de Biochimie, Nice.

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Biochem. biophys. Res. Comm. **88**, 950; 1979) a-cobratoxin (Walkinshaw *et al. Proc. 6th Int. Symp. on Animal, Plant and Microbiol Toxins* in the press) and suggestions that regions on these molecules mimic agonists (Low, *Handbook exp. Pharmacol.* **52**, 213; 1979) and curarare-like antagonists (Hider, *Adv. Cytopharmac.* **3**, 147; 1979) were presented at the workshop.

As a result of the specificity of these toxins for AChR, they are used for the isolation of the receptor by affinity chromatography and for monitoring AChR levels and turnover rates in muscle and myasthenic patients (Dolly, *Int. Rev. Biochemistry* **26**, 257; 1979).

The sodium channel

Until recently knowledge of the sodium channel has been largely based on electrophysiological data; however, with the discovery of a number of specific toxins this membrane-bound protein has now become susceptible to biochemical study. T. Narahashi (Northwestern University, Chicago) indicated that this large molecular complex appears to be more complicated than AChR, a fact possibly related to its ability to sense membrane potential. Three major toxin-binding sites are being used to characterise the sodium channel — the ionophore site that binds tetrodotoxin (from puffer fish), together with the two peripheral sites, one which opens sodium channels when occupied by the lipophilic toxins, veratridine (a plant alkaloid) and batrachotoxin (from the Colombian frog), and one which blocks sodium inactivation when occupied by polypeptide toxins isolated from sea anemone and scorpion species. An important limitation to the use of lipophilic toxins emerged when W. Ulbricht (Physiologisches Institute, Kiel) warned that the non-specific detergent properties of veratridine begin to predominate above 10^{-4}M.

W.A. Catterall (University of Washington, Seattle) described his two-state model of the channel (*Adv. Cytopharmac.* **3**, 305; 1979) and on the basis of toxin-binding studies suggested a molecular interpretation of the classical Hodgkin-Huxley model of electrical excitation of squid giant axon. Hodgkin and Huxley showed that the activation of sodium channels depends on the value of two voltage-dependant variables (m and H) according to the expression m^3h and Catterall has demonstrated that the ratio of tetrodotoxin and scorpion toxin sites is approximately 3:1 (Catterall & Morrow *Proc. natn. Acad. Sci. U.S.A.* **75**, 218; 1978). This could account for the original m^3h empirical finding. Lazdunski presented recent work on the development of highly radiolabelled derivatives and photoaffinity labels of tetrodotoxin and sea anemone toxin (Lazdunski *et al. Adv. Cytopharmac.* **3**, 353; 1979). It is likely that these compounds will have an important part to play in the elucidation of the sodium channel structure. Indeed the sea anemone toxins may well prove to be suitable for affinity chromatography of the sodium channel. J.C. Fontecilla (University of Alabama) announced the 3-D backbone structure of a scorpion neurotoxin (*Centruroides sculpturatus*), the first to be determined by X-ray crystallography. This important finding, together with the controversy concerning voltage-dependent binding make it likely that the polypeptide toxins will be a focus of attention for the next few years.

Surprisingly, no toxins have yet been found with high affinity for potassium or calcium channels.

Neurotoxic phospholipases

The third major topic of the meeting centred on snake venom phospholipases, and it was fitting that H. Fraenkel-Conrat (University of California, Berkeley) should present a paper as he was the first biochemist to purify a snake toxin (*Berichte* **71**, 1076; 1938). This protein, crotoxin, was isolated from the South American rattle snake and was found to be a phospholipase. His original isolation method is still used today.

Many snake venom phospholipases are extremely potent toxins with a high affinity for neuromuscular junction membranes. P. Strong (University College, London) described the biphasic behaviour of β-bungarotoxin (from the many banded krait) on presynaptic membranes. After an initial binding response which results in a decline of acetylcholine release, a secondary response expressed by an elevated level of release occurs over a 2-hour period. This latter response finally results in the irreversible inhibition of transmitter release. The initial binding response is independent of phospholipase activity, in contrast to the second effect which is completely dependent on such activity (Kelly, von Wedel & Strong *Adv. Cytopharmac.* **3**, 77; 1979). A plausible explanation for this phenomenon is that the toxin initially binds selectively to the plasma membrane and subsequently the phospholipase-active portion of the toxin promotes hydrolysis of the membrane phospholipids. The nature of this specific binding site is not known, although B. Howard (University of California Medical School, Los Angeles) presented evidence to suggest that it would be the Ca translocating system.

One confusing aspect of neurotoxic phospholipases is that many have more than one protein subnit. They all seem to possess a phospholipase unit homologous to porcine pancreatic phospholipase, and this catalytic portion is connected by means of disulphide bonds or electrostatic/hydrophobic interactions to a second nonenzymatic unit. C. Bon (Institut Pasteur, Paris) suggested that the nonenzymatic subunit of crotoxin, which possesses a net negative charge, enhances the pharmacological efficiency of the positively charged phospholipase component by preventing its absorption to nonsaturable binding sites, restricting its binding to specific sites (Bon & Jeng *Adv. Cytopharmac.* **3**, 231; 1979). A similar mechanism has been suggested by Hendon and Tu (*Biochim. biophys. Acta* **578**, 243; 1979). However, this is unlikely to be a general mechanism for neurotoxic phospholipases as, for instance, the net charge of the nonenzymatic portion (B-chain) is 5^+, whereas that of the phospholipase portion (A-chain) is close to zero. An alternative function for the B-chain is implicated for this molecule. Surely it is significant that the B-chain is homologous to the Kunitz-type of proteinase inhibitors (Kondo, Narita & Lee *J. Biochem.* **83**, 101; 1978) and that other such homologues have been isolated from venoms of the Elapidae (Joubert & Strydom *Eur. J. Biochem. 87*, 191; 1978).

It is becoming clear that many of the membrane proteins in excitable tissue undergo conformational changes which can be explained in terms of simple allosteric models. As membrane bound trypsin molecules render lipid bilayers permeable to ions when exposed to typical trypsin substrates it is not difficult to envisage how receptors of the type described by Barnard and Changeux could have evolved from water-soluble enzymes.

R.C. Hider is in the Department of Chemistry, University of Essex.

Invertebrate respiratory proteins

from E. J. Wood

Because the first X-ray and sequence studies are now appearing, rapid advances can be expected in the next few years in our knowledge of the structure and function of invertebrate respiratory pigments. These proteins, which include the haemocyanins, the erythrocruorins and chlorocruorins, and the haemerythrins (see Hendrickson, *TIBS*, May 1977 for a brief review), were discussed at a recent EMBO workshop in Tours*.

Haemocyanins are non-haem copper proteins which exist as giant aggregates dissolved in the blood of molluscs and arthropods. Mollusc haemocyanins possess very large subunits, and in gastropods, for example, 20 such subunits are assembled in a helical fashion to produce a 9 million molecular weight structure with a 'collar' at each end. The subunits are thought to be single polypeptide chains each consisting of eight

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*The EMBO workshop 'Comparative Study and Recent Knowledge on Quaternary Structure and Active Sites of Oxygen Carriers and Related Proteins' was organised by J. & F. Lamy at the University of Tours, France, on 20–24 August.

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covalently linked oxygen-binding moieties of molecular weight around. 50,000, usually referred to as ‘oxygen binding domains’, in a string. It has proved difficult to determine the exact molecular weight of this basic subunit by physical methods, and SDS-gel electrophoresis does not seem to give reliable values for apparent molecular weights in this range (molecular weight 300,000). Preliminary studies on the biosynthesis of mollusc haemocyanins seem to show that the product of mRNA translation is indeed very large (E. J. Wood, University of Leeds).

From proteolytic digestion studies information on the order of the different types of domain in the string is emerging. (R. Lontie, Katholeike Universiteit, Groningen). Several of the domains are now available in a reasonably pure state and immunological, as well as spectral criteria are used to distinguish them. It seems likely that they differ functionally which may explain certain heterogeneities in functional properties of these haemocyanins.

Arthropod haemocyanins differ from molluscan ones in a number of respects including electron microscopic appearance and their comparatively small subunit polypeptide chains which contain single oxygen-binding sites. Up to seven types of subunits are needed in the assembly of the native molecule in scorpion and *Limulus*, and J. Lamy (University of Tours) has shown how useful immunological techniques are in identifying and quantitating subunits, as well as for helping in the phylogenetic classification of groups of arthropods. Certain subunits share both immunological and assembly correlates in a number of species, and subunit stoichiometry is becoming better understood. Reassembly studies have shown, for example, that for *Eurypelma* haemocyanin, three different types of subunit are needed for the formation of a 16S hexamer, five different for a 24S dodecamer, and all seven for a stable 37S aggregate or ‘24-mer’ (which is the native form). Sequence studies are already showing some homologies between subunits in haemocyanins from *Eurypelma* (a tarantula) and *Androctonus* (a scorpion).

Complete ignorance of the protein structure has so far prevented a description of the changes when O_2 binds to the active site of haemocyanins. A simple model for allostery in haemocyanins was proposed in which Cu—Cu distances affect the strength of O_2 binding and the rate of O_2 dissociation (C. Bonaventura, Duke University Marine Laboratory). Oxygen binding by haemocyanins from both arthropods and molluscs shows certain common features. The discovery that deoxy and carbonmonoxy-haemocyanins (H. Kuiper, University of Rome) have different fluorescence spectra will allow the CO derivatives to be studied in much more detail than has hitherto been possible.

The binding of O_2 by haemocyanin, which is often cooperative, can be described, approximately at least, by a two-state model in which allosteric ligands may bind preferentially to the T or the R state. M. Brouwer (Groningen) has studied the binding of O_2 by *Limulus* haemocyanin and concluded that the ‘switch-over’ in state occurred (mostly) as the fourth O_2 was added. However, binding of the allosteric ligands Cl^- or H^+ can change this. The kinetics of the R-T transition are under investigation and it is suggested that the size of the allosteric unit may be correlated with the rate of the conformational change (M. Brunori, University of Rome).

The relationship between the functional properties of haemocyanins and the haemolymph concentrations of such allosteric ligands (as well as of Ca^{2+} and Mg^{2+} which can control the aggregation state of the molecules) deserves more study than it is receiving at present. The fact that many haemocyanin-containing animals have little or no ability to regulate the ionic composition of their blood may lead to a consequential limitation of O_2-transporting properties, or there may be an important physiological adaptation reflected in the effect of these so-called allosteric effectors on the functional properties of haemocyanin (C. Mangum, Williamsburg).

Several groups are working on the big haemoglobins (or erythrocruorins) of annelids and on the rather similar chlorocruorins. Although the molecules occur in haemolymph as enormous aggregates (molecular weights of up to 4×10^6) they have much smaller subunit polypeptide chains than mollusc haemocyanins. However, general rules about the number of subnits and their sizes have yet to emerge, and it has been difficult to agree on the minimal molecular weight per haem based on chemical determinations. One subunit polypeptide chain from the earthworm, *Lumbricus*, haemoglobin has now been sequenced (Riggs *Fed. Proc.* **38**, 343; 1979) and has 157 residues corresponding to a molecular weight of 17,500. The sequence shows definite homologies with the ß-chain of human haemoglobin. Uncertainties about size and shape of subunits have so far prevented formulation of a definitive model for the native molecular aggregate.

Although the ‘annelid type’ of haemoglobin is common, even being found in a deep-sea tube-dwelling worm from a hydrothermal vent, as reported by N. Terwilliger (Charleston, Oregon), many other types are known. These range from the monomeric (and sometimes dimeric) chironomid haemoglobins, for which there is much sequence information and which are like myoglobin (T. Kleinschmidt, University of Munich), through what might be called medium-sized haemoglobins from *Artemia* (F. De Voeght, Wilrijk, Belgium), branchiopods (E. Ilan, Tel-Aviv) and planorbid snails, to those even bigger than the annelid type such as that from a clam, *Cardita*, reported by R. Terwilliger to have a molecular weight of 8×10^6. In contrast to the annelid type of haemoglobin many of these seem to have very large (presumably multidomain) polypeptide chains.

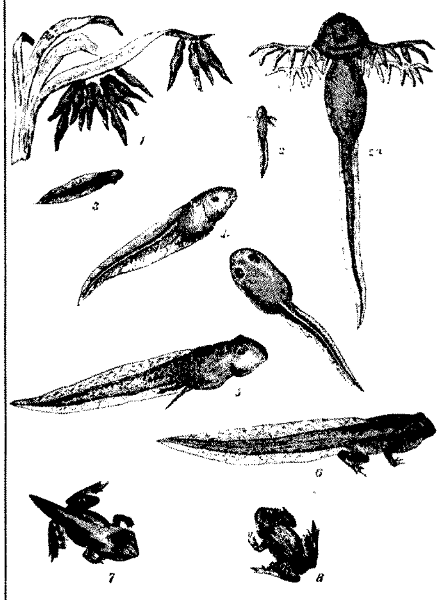
The third group of invertebrate respiratory pigments comprises the nonhaem iron proteins, the haemerythrins. Very much more is known about these proteins which have only a limited biological occurrence, than about the haemocyanins or invertebrate haemoglobins although admittedly they have a much simpler structure. The coelomic forms (tri, tetra and octomers) have a polypeptide chain of 113 amino acids while the monomeric muscle myohaemerythrin has 118, but there are nevertheless considerable homologies. The coelomic form has four major helical regions (A—D): myohaemerythrin differs in having five extra residues in the C-D corner (W. Hendrickson, Washington). It seems that the polymeric forms possess different subunits, but the differences are minor. The iron site is prominent and resonance Raman studies (D. Shriver, Evanston) have given valuable clues to the mode of oxygen binding. ☐

E. J. Wood is in the Department of Biochemistry, University of Leeds.

100 years ago

TAILS

The tadpole is at first a singular object. It consists of a hed and body indistinguishably united in one rounded all, from behind which a long and slender tail projects. The creature may be said to be indeed at first "all head and tail."



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review article

Crown gall disease

Martin Drummond
ARC Unit of Nitrogen Fixation, University of Sussex, Falmer, Brighton, UK

Agrobacterium tumefaciens *induces tumours in dicotyledonous plants by transferring part of a large bacterial plasmid to the eukaryotic cell. As well as disrupting control of cell division, the transferred DNA determines the synthesis in transformed tissue of novel amino acid compounds which serve as specific substrates for the bacterium. It may prove possible to exploit such DNA transfer for the genetic engineering of higher plants.*

CROWN GALL is a plant tumour caused by *Agrobacterium tumefaciens.* It was first described by Aristotle, first assigned a bacterial aetiology by Smith and Townsend[1], and first proved to be a true oncogenic transformation by White and Braun[2], who showed that crown gall tissue cultured in the absence of the bacterium retained its tumorous properties. The disease occurs in a remarkably wide variety of higher plants[3] but can only be induced in freshly wounded tissue[4]. Oncogenic strains of the bacterium contain a large plasmid, part of which is transferred to the plant cell during oncogenic transformation and maintained there indefinitely[5]; to this extent crown gall resembles the virally induced animal tumours. Transformed tissue is distinguishable by its ability to form an overgrowth when grafted onto a healthy plant, its capacity for growth in tissue culture without added plant hormones[6], and its synthesis of 'opines', unusual derivatives of basic amino acids not found in normal tissue[7-10]. The study of crown gall may help the understanding of carcinogenesis and should be important in elucidating the control of gene expression in higher plants. The possibility of using *Agrobacterium* plasmids as vehicles for genetic engineering in higher plants is also of interest.

Understanding the disease at the molecular level only became possible recently with the discovery that all oncogenic strains of *Agrobacterium* contained large plasmids of about 150–230 kilobases[11], which proved to be essential for tumorigenesis. Hamilton and Fall[12] had shown that one bacterial strain became non-tumorigenic when cultured at 37 °C, and this was shown to be invariably associated with plasmid loss[13-16]. When other *Agrobacterium* plasmids were introduced into the cured strain by conjugation or transformation it recovered oncogenicity, confirming the involvement of the plasmid in oncogenesis[16-21]. Not all large *Agrobacterium* plasmids confer the ability to induce tumours; those that do are designated Ti (tumour-inducing) plasmids. None is smaller than 140 kilobases. So far relatively few genetic markers have been ascribed to them.

The catabolic function of Ti plasmids

The metabolism of opines is a central feature of crown gall disease. Axenic crown gall tissue synthesises opines which can serve as a carbon or nitrogen source for oncogenic agrobacteria. Morel and coworkers noted that the nature of the opine produced is determined not by the host plant but by the bacterium, and is invariably the one catabolised by the inducing strain[22,23]. They interpreted this correlation as evidence for DNA transfer from the bacterium to the plant cell, and suggested that the enzyme catabolising a given opine in the bacterium was identical with that producing it in tumour tissue. In fact two different enzymes are involved, distinguishable both genetically and biochemically[8,24-28]. The enzyme responsible for bacterial catabolism is referred to as an opine oxidase, while that in the plant is termed a synthase. Both are probably plasmid-encoded, although this has not yet been demonstrated unequivocally. With a single exception, the Ti plasmids isolated so far code for the synthesis and breakdown of either octopine or nopaline but not both. The substrate specificity of synthases and oxidases is, however, not exact and both octopine and nopaline tumours contain a characteristic family of opines, comprising analogues of octopine or nopaline (see Fig. 1), which can also support the growth of the inducing strain of *Agrobacterium*[29-34]. Most octopine tumours also contain massive amounts of the recently discovered opine agropine, which is probably the product of a separate synthase, as it is absent from some tumours that do contain octopine (J. L. Firmin, personal communication), and stereochemically is quite unlike octopine[35]. The finding that the plasmid pTi542 codes for agropine metabolism alone bears out this supposition (J. Tempé and J. L. Firmin, personal communications). Three distinct plasmid-encoded permeases exist for nopaline, octopine and agropine[35,36]. The expression of oxidases and permeases by octopine and nopaline determining plasmids has been shown to be coordinately controlled, both enzymes being inducible by their particular substrate[37]. In some strains, the catabolism of arginine, a breakdown product of both octopine and nopaline, is a plasmid-borne trait which is also inducible by the appropriate opine[38].

Ti plasmids promote bacterial conjugation[39,40], and the genes required for their transfer (*tra* functions) are closely associated with opine metabolism, being inducible by the characteristic opine[41,42]. This makes good teleological sense, as in the presence of its substrate the catabolic plasmid will spread rapidly through a mixed population of oncogenic and non-oncogenic bacteria. The three classes of regulatory mutant that can be isolated are most simply explained by postulating separate operons for plasmid transfer and opine catabolism, controlled by the same repressor[43-45].

Tumorigenesis is almost always accompanied by opine synthesis, but as one can isolate mutant plasmids that give tumours failing to produce any opine, such synthesis cannot be necessary for tumour maintenance[45,46]. However, there is some evidence that opines enhance tumour growth[47,48]. Perhaps the

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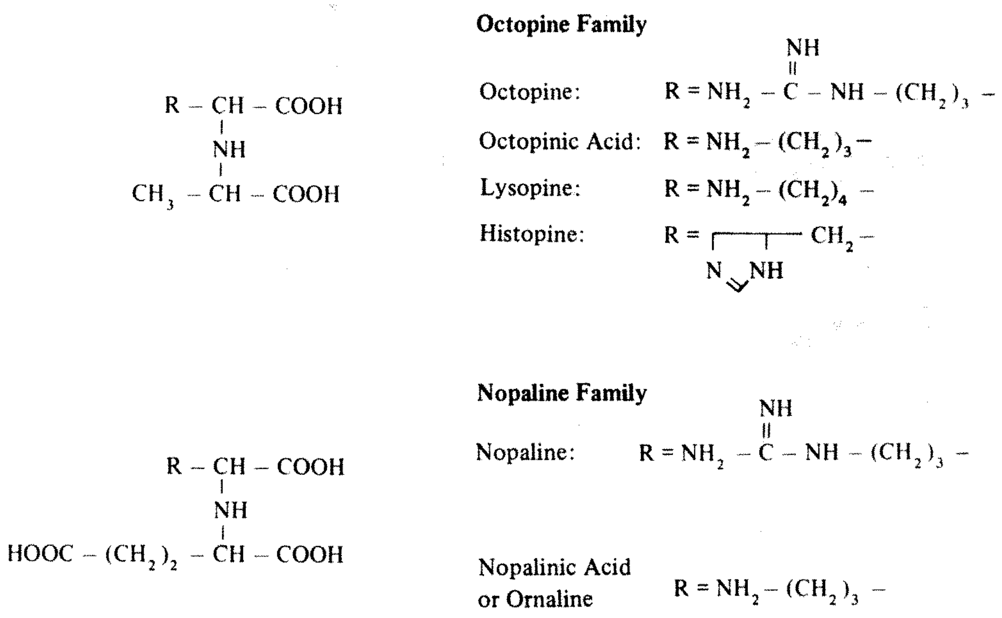
Octopine Family
R – CH – COOH
NH
CH3 – CH – COOH
Octopine: R = NH2 – C(=NH) – NH – (CH2)3 –
Octopinic Acid: R = NH2 – (CH2)3 –
Lysopine: R = NH2 – (CH2)4 –
Histopine: R = (imidazolyl) CH2 –
Nopaline Family
R – CH – COOH
NH
HOOC – (CH2)2 – CH – COOH
Nopaline: R = NH2 – C(=NH) – NH – (CH2)3 –
Nopalinic Acid or Ornaline R = NH2 – (CH2)3 –

Fig. 1 Structural formulae of opines synthesised by crown gall tumours.

most plausible rationale for the presence of opines in tumour tissue is that they provide a specific ecological niche for the oncogenic bacterium[43]. As only bacteria capable of using opines as carbon and nitrogen sources can exploit the niche, the plasmid thereby generates selection pressure for itself as well as promoting its own transfer through the population. The logical link between opine production and uncontrolled cell division is that the plasmid need only transform a single cell to procure a relatively abundant supply of its opine substrate. It is quite conceivable that other plasmids are also capable of introducing some of their genetic information into eukaryotic cells but go undetected because they lack the more conspicuous oncogenic phenotype.

The opine strategy of the Ti plasmids can be seen as an example of altruism at the molecular level. Altruism is a property of a gene which reduces the darwinian fitness of the individual while enhancing the reproductive potential of the population to which it belongs. T-DNA in the plant cell cannot ultimately enhance its own chances of survival as it must perish with the somatic tissues of the plant, but its expression increases the chances of replication of T-DNA that remains part of the bacterial replicon.

Models of tumorigenesis

A complete description of the crown gall transformation must explain how plasmid DNA enters the plant cell, how it manages to be replicated in the eukaryotic genome and how it exerts its characteristic physiological effects. Much of this is now known, but a full description involves some speculation.

The absolute requirement for a wound for tumorigenesis in the whole plant must be accounted for. Wounding might be necessary to expose bacterial attachment sites on the plant cell surface. A lipopolysaccharide element of the bacterial outer membrane is known to adsorb to specific sites on the plant cell wall[49,50]. An alternative explanation postulates a need for cell division associated with wound healing for successful oncogenesis. This is more consistent with the finding that only if a lesion is inoculated with *Agrobacterium* before the first wave of cell division—up to 2 days after wounding—does oncogenesis proceed as usual[51,52].

The formal resemblance of the plasmid transfer phase of oncogenesis to bacterial conjugation may be reflected at molecular level. Whether the entire Ti plasmid enters the plant cell is not known, but both transformation and conjugation are temperature sensitive with critical temperatures around 30 °C, suggesting that one or more of the oncogenic (*onc*) and transfer functions could be identical[42,53]. However, mapping of the *tra* and *onc* genes in both octopine and nopaline plasmids has not yet revealed any overlap of these functions[45,46].

As well as the genes for plasmid transfer, octopine and nopaline Ti plasmids presumably share at least some of the genetic information involved in oncogenesis. The extent and distribution of DNA sequences conserved on all Ti plasmids is thus relevant to the mechanism of transformation. Octopine plasmids are all very similar in their restriction patterns and

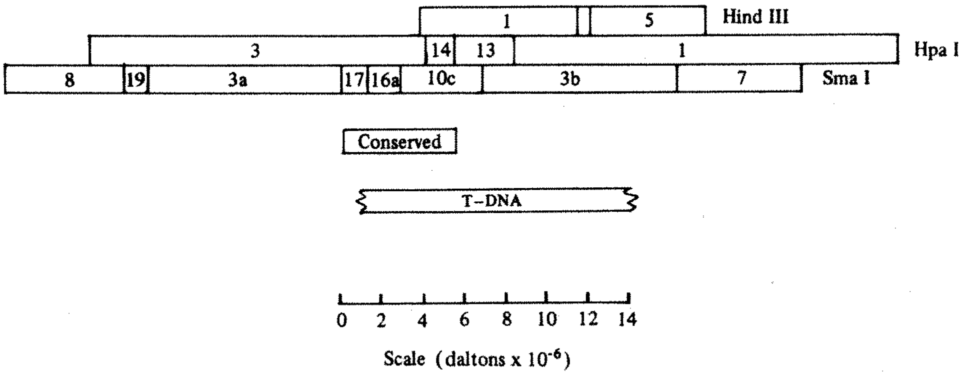

Fig. 2 Section of the physical map of the typical octopine plasmid pTiB6-806 showing fragments detected in tumour tissue (T-DNA) and the DNA which is stringently conserved on all Ti plasmids. Redrawn from Chilton *et al.*[61].

sequence homology[54,55] but are only 20–60% homologous with various nopaline plasmids[56], which themselves form a much more heterogeneous group[52]. Thus if one assumes there has been no divergence in the *onc* functions, only 20% of the information content of the Ti plasmids can be involved in tumorigenesis. This would raise the question of why the plasmids are invariably so large—perhaps the *onc* genes have diverged. It is clear that the opine determinants have done so, maybe to exploit host plants containing different major constituents of a particular opine, and divergence of the *onc* genes in response to host biochemistry is entirely consistent with the plasmid-borne variations in host range which have been observed[57].

Chilton *et al.*[5] demonstrated the presence of octopine plasmid DNA in a cloned tobacco tumour by using an entire set of plasmid restriction fragments as probes in a series of solution hybridisation experiments. They found $\sim$20 copies per cell of a 6-kilobase segment representing about 3% of the plasmid. Such stretches of plasmid DNA, designated T-DNA, are probably integrated to the plant genome[46]. The mechanism of integration is unknown, but may well differ from that of prokaryotic transposons as stretches of T-DNA derived from a single cell can vary in length[58].

Experiments with restriction fragments containing T-DNA of an octopine plasmid show that the left hand end of the octopine T-DNA and sequences extending as far as its left-hand boundary are highly conserved on all Ti plasmids[59,60] (see Fig. 2). Heteroduplexes of this region show 3–5% base mismatching, markedly lower than the 9–12% mismatching observed for DNA sequences conserved elsewhere on the plasmid[56,60]. Experimental integration of the plasmid RP4 into its area of highly conserved sequences renders a Ti plasmid non-oncogenic[59]. This DNA segment must therefore function in oncogenesis. The greater part of the conserved region is found in all tumour lines that have been examined and is probably sufficient for tumour maintenance[46,58,61]. A 6-kilobase *Sma*I restriction enzyme fragment common to the majority of Ti plasmids encompasses about half of this DNA[60,61] (Fig. 2). The T-DNA to the right of the stringently conserved segment on the octopine plasmid has little or no discernible homology to the corresponding region on the various nopaline plasmids[59,60]. Restriction mapping of insertion mutants of both octopine and nopaline plasmids indicates that this region either contains the opine synthase gene or at least contains the gene that controls opine synthesis by the host[46].

There are two possible ways in which T-DNA could exert its physiological effects. Oncogenesis could result simply from the insertion of T-DNA onto host genes crucial to the control of cell division. The presence of T-DNA in variable copy numbers per cell, and the phenomenon of phenotypic reversion (see below) are, however, easier to accommodate in a model involving a specific oncogenic gene product, analogous to the T-antigen of SV40-transformed rodent cells[62].

At least part of the T-DNA is transcribed in the plant cell. If ^{32}P-labelled RNA from an octopine-producing tumour is hybridised to Ti plasmid restriction fragments that have been transferred to cellulose nitrate paper by Southern's method[63], it binds strongly to *Sma*I fragment 3b, which includes the right-hand boundary of the T-DNA[64]. The labelled RNA is polyadenylated[65] and must include the transcript of the octopine synthase gene if that gene is plasmid encoded. In the nopaline tumour examined by Yang *et al.*[58], there appear to be at least two plasmid RNA species present, indicating that T-DNA sequences other than those coding for nopaline synthase are also transcribed. At least one of these RNA species appears to be from the highly conserved T-DNA, and transcripts from this region have also now been detected in octopine tumours[65,66]. The conserved DNA could encode the putative oncogenic gene product.

Hormone imbalance is strongly implicated in the aetiology of crown gall disease. Cytokinins, which promote cell division, are abundant in crown gall tissue, as are auxins, a class of plant

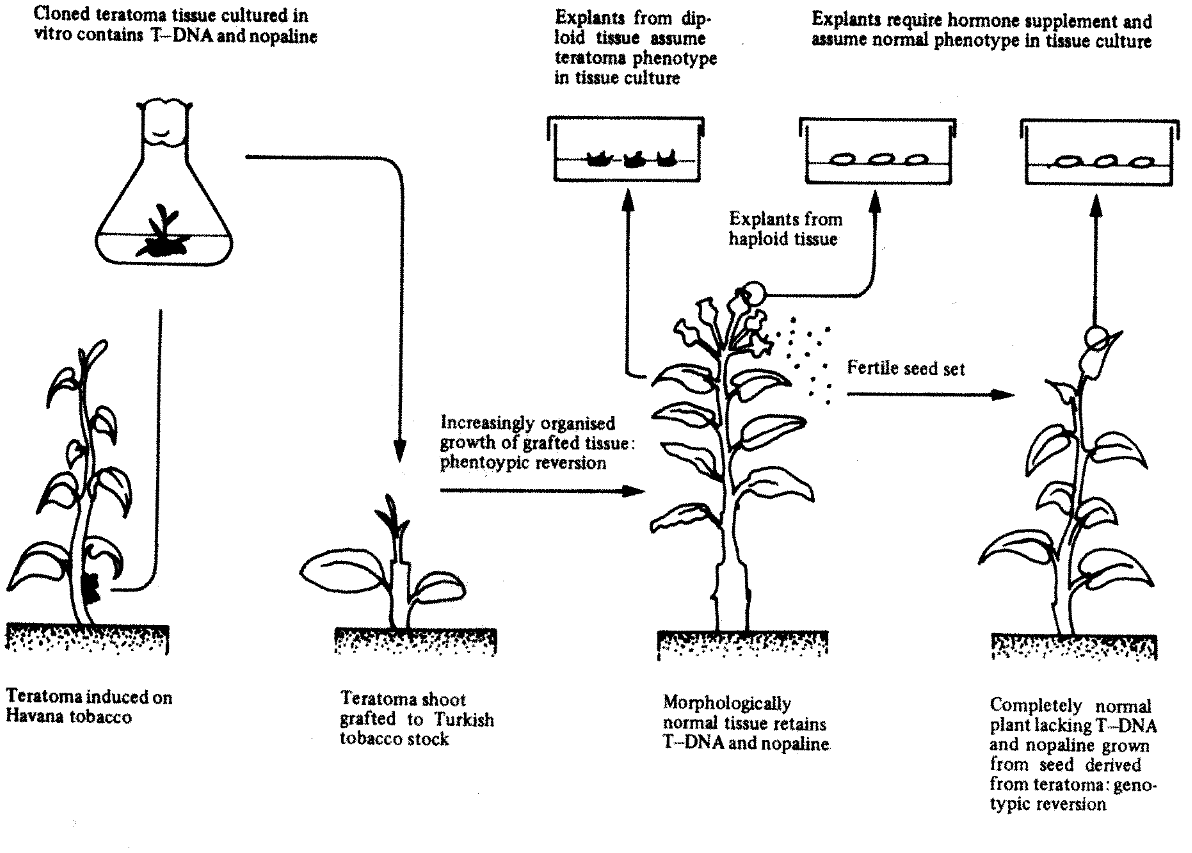
Cloned teratoma tissue cultured in vitro contains T–DNA and nopaline
Explants from diploid tissue assume teratoma phenotype in tissue culture
Explants require hormone supplement and assume normal phenotype in tissue culture
Explants from haploid tissue
Fertile seed set
Increasingly organised growth of grafted tissue: phentoypic reversion
Teratoma induced on Havana tobacco
Teratoma shoot grafted to Turkish tobacco stock
Morphologically normal tissue retains T–DNA and nopaline
Completely normal plant lacking T–DNA and nopaline grown from seed derived from teratoma: genotypic reversion

Fig. 3 Reversal of the crown gall transformation.

hormones which stimulate cell enlargement[67,68]. Untransformed tissue cultured *in vitro* does not contain physiologically effective levels of either hormone[6]. Furthermore, plant tumours caused by Black's wound tumour virus as well as those occurring spontaneously in interspecific hybrids of *Nicotiana* also contain abnormally high levels of these hormones[69,70]. Such hormone autotrophy might thus be a sufficient condition for plant neoplasia in general.

Reversion

Crown gall tumours vary considerably in their morphology, some producing recognisable stems or roots while others remain a chaotic mixture of cell types. Ti-plasmid-borne genes determine morphology to some extent[71]. Nopaline tumours typically organise stem primordia on their surfaces, in which case they are referred to as teratomata. When teratoma shoots are grafted to the apex of a normal plant they are forced into rapid growth and gradually regain normal morphology[72,73], a phenomenon termed phenotypic reversion (see Fig. 3). The use of cloned teratoma tissue removes the possibility of selection of untransformed cells, and morphological markers serve to distinguish clearly the scion from the stock. The redifferentiated plant contains T-DNA[58], synthesises nopaline[74], and reverts to the tumour phenotype when explanted into tissue culture[75]. This suggests that phenotypic reversion has an epigenetic basis, in which case studying it should be fruitful in understanding control of gene expression in higher plants.

The crown gall transformation may also be reversed at the genotypic level. Redifferentiated teratoma shoots sometimes flower and set viable seed. Plants grown from this seed lack T-DNA[58], do not synthesise any opine and require a hormone supplement in tissue culture[75]; their recovery is apparently complete. This recovery appears to occur during meiosis, as haploid tissues derived from anther cultures also fail to synthesise nopaline and show the normal hormone requirement in culture. Such complete recovery occurs only very rarely in diploid tissues[75]. Loss of T-DNA at meiosis could occur in one of three ways: (1) haploid progeny carrying T-DNA are not viable (the integration of T-DNA could cause recessive mutations); (2) chromosomes bearing such insertions are specifically excluded from the progeny; or (3) there exists some machinery in the meiotic cell which can excise extraneous material from the genome (controlling elements in maize survive meiosis, however). None of these possibilities can be excluded at present.

Genetic engineering with Ti plasmids

It should be possible to use Ti plasmids as vectors to insert a chosen DNA sequence into the genome of any dicotyledonous plant. The phenotypic reversion described above shows that this need not disrupt normal differentiation, but genotypic reversion may limit the technique to plants that can be propagated vegetatively. The difficulties involved in introducing a bacterial gene into a crop plant and having it expressed appropriately are daunting. For example, simply inserting *nif* (nitrogen fixation) genes into the potato genome would be useless, as to be of value their expression must be subjected to eukaryotic control and some system devised to protect the nitrogenase from oxygen poisoning. More immediate applications of the technique may involve restoring to their original genome plant DNA sequences which have been modified *in vitro*, say to increase the lysine content of a storage protein.

The first step in any such project would be to identify and probably isolate the DNA sequence encoding the desired trait. This must then be inserted into the T-DNA. To introduce foreign DNA sequences into a prokaryotic cloning vehicle it is sufficient simply to cut the vehicle into one or two pieces with a suitable restriction enzyme, then reassemble it with the 'passenger' in place. The Ti plasmids, however, being so large, are extensively cleaved by the available restriction enzymes, and the chances of reassembling a functional whole with the passenger in the desired site are remote. This difficulty could possibly be obviated by deleting unnecessary segments of the plasmid or by modifying restriction sites where cleavage is not wanted. An alternative would be to use a restriction enzyme such as *Hph*I that cleaves at some distance from its recognition site[76]; the 'sticky' ends generated at each cut are then unique, which guarantees accurate reassembly of the plasmid. The passenger could be inserted into an isolated fragment containing T-DNA before the whole is reassembled.

However, it might be possible to avoid manipulating DNA *in vitro* altogether. Schell and his collaborators allowed the transposable element Tn7, which codes for streptomycin resistance, to hop into the T-DNA of a nopaline plasmid. The modified T-DNA was then introduced into the tobacco genome, where it was detected using Southern's[63] technique. The chosen genetic trait is unlikely to be a transposable element, of course, but one could attempt to make it so. Some transposons have been shown to be flanked by insertion sequences[77,78] which may well be sufficient to promote transposition[79]. It should be possible to construct a bacterial plasmid having an insertion sequence at either end of the DNA sequence one wishes to transpose into the T-DNA.

Whether or not it proves possible to exploit the peculiar properties of the Ti plasmids for genetic engineering, work on crown gall disease will continue to make an important contribution to our understanding of the molecular biology of higher plants.

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articles

Crust of oceanic affinity in Iceland

I. L. Gibson Iceland Research Drilling Project (Edited by I. L. Gibson*)
Department of Land Resource Science, University of Guelph, Ontario, Canada

Evidence for subsidence of at least 2 km during crustal formation and the absence of an increase in minor intrusion density with depth in the lower 2 km are the major results of the field study of a 3-km vertical section of Icelandic crust.

DURING the past six years major efforts have been made to determine the structure, composition and mode of formation of the oceanic crust. Such investigations are, however, severely hampered by the great depth of water in the ocean basins and the difficulty of deep drilling in the basaltic layer of the crust. An alternative approach is to study elevated mid-ocean ridge segments such as Iceland. Although not underlain by typical oceanic crust, Iceland forms a part of the Reykjanes–Kolbeinsey section of the Mid-Atlantic Ridge and contains a record of eruptive processes at a spreading axis. It is also well exposed and easily accessible for study. A detailed investigation of the Icelandic crust should provide important information on the processes of crustal construction, hydrothermal alteration and magmatic evolution of one part of the Mid-Atlantic Ridge.

The first results from a continuously cored, 1,919-m deep borehole drilled in eastern Iceland, forming the lower part of an intensely sampled 3-km section, are reported here. The section is entirely within seismic layer 2. Overall core recovery was 99.7%. Hole diameter was 7.6 cm down to 1,350 m, then 6.0 cm to the bottom.

The structure of eastern Iceland is relatively simple and consists of a thick succession of deeply dissected Neogene flood basalts which dip gently westwards towards and beneath the Neovolcanic zone of North-east Iceland. Walker[1] showed that the lava pile consists of a series of overlapping groups of flows that thin progressively up-dip. Lava dip decreases from 7–10° near sea level to 2–3° near the 1,000-m summit level. The nature of the regional zeolite grade metamorphism suggests that the basalt pile had a finite limit about 1.5 km above sea level where the intensity of dyke injection approached zero and the lavas were horizontal and unzeolitised.

The drill site (lat 65° 01′ N, long 14° 21′ W) is located near sea level, 8 km east of the centre of the Thingmuli central volcano, at the head of Reydarfjordur in a geologically and geophysically well known area[2–6], where crustal dilation by north–south basaltic dykes is 10% (Fig. 1). Final site selection was on the basis of unpublished gravity data (G. Palmason, personal communication) which were used to identify a location within a large area of anomalously high temperature gradient ($\sim$80 °C km^{-1} (ref. 7)) where the regional gravity anomaly was of average value.

Lava stratigraphy

The combined exposed and drill-hole 3-km section consists of subaerial lava flows, with thin intercalated pyroclastic and volcaniclastic sedimentary horizons, all cut by minor intrusions (Fig. 2*a*, *b*). In the drilled sequence minor intrusions comprise 40% of the core and cut out large sections of flow stratigraphy.

Six minor faults with displacements up to 50 m are present in the exposed section and similar faults probably cut the drilled succession, although no obvious stratigraphic repetitions were observed. Regional mapping has indicated the absence of major faults in the area[2].

All thickness values quoted for both the exposed and drilled sequences are vertical thicknesses. The deviation of the hole from the vertical was measured by acid tests at 100-m intervals and was usually much less than the maximum value of 7°.

The observed dip in the cliff section varied from 2° at 1,000 m elevation to 10–14° at sea level. Dips measured in the drill core are scattered but suggest that the observed increase with depth continues until a value of about 20° is reached at about 500 m below sea level, below which little further increase occurs.

Examination of the drill core in the field showed that the flows are predominantly subaerial fine-grained aphyric tholeiitic lavas characterised by reddened rubbly flow tops and massive to weakly flow-banded grey-green interiors, corresponding to the tholeiites and basaltic andesites of Walker[2]. Notable exceptions include a 12-m thick Icelandite flow from 1,083 to 1,095 m (unit 185.5) and a 31-m thick welded ash-flow tuff sequence from 920 to 951 m (units 157.2, 161.1 and 162.1).

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* The members of the scientific party of the Iceland Research Drilling Project are listed at the end of the article.

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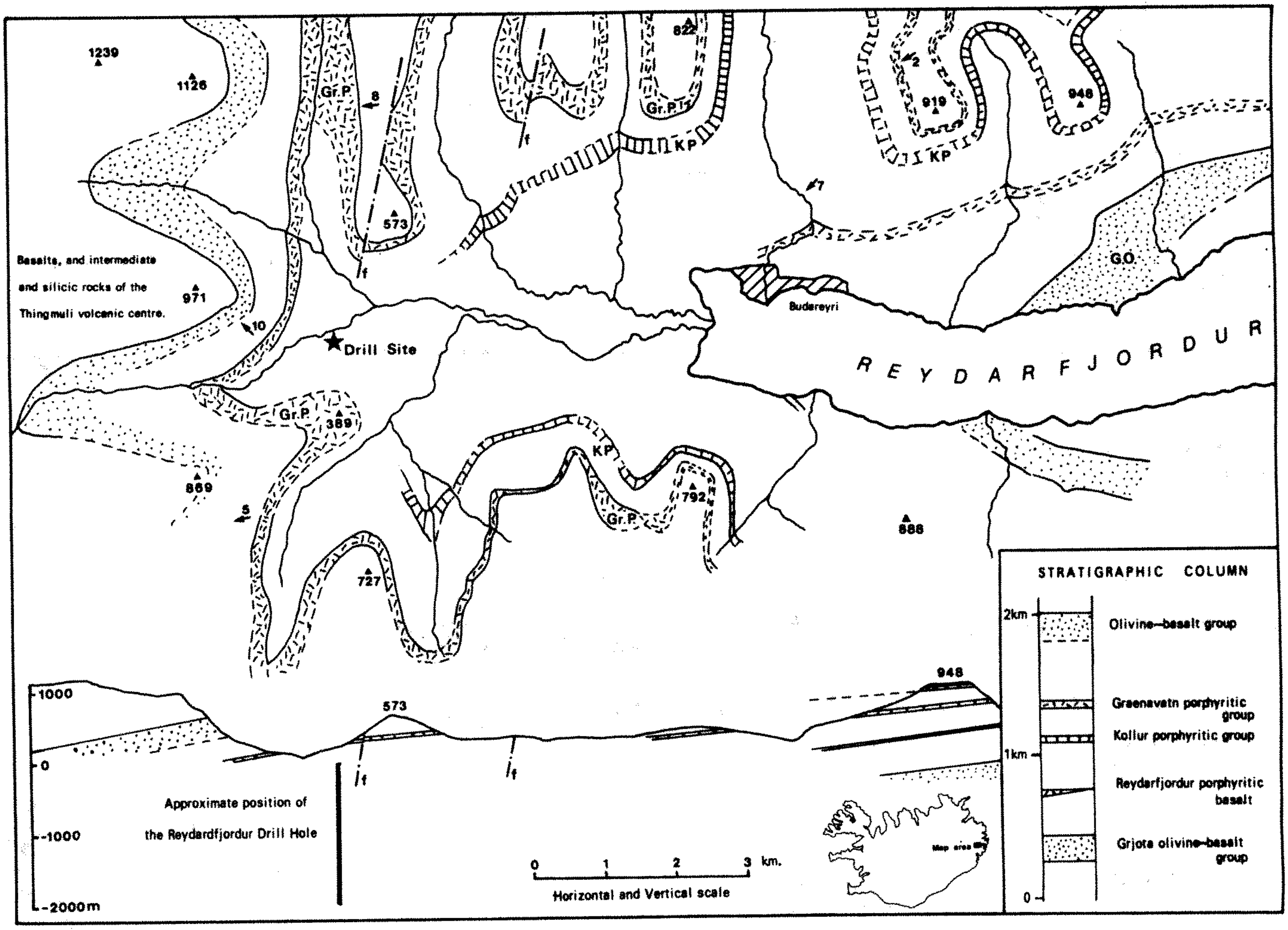
1239
1126
Gr.P
8
822
Gr.P
KP
2
919
KP
948
573
7
Basalts, and intermediate and silicic rocks of the Thingmuli volcanic centre.
971
10
Drill Site
Budareyri
G.O.
REYDARFJORDUR
Gr.P
389
KP
792
Gr.P
869
5
888
727
STRATIGRAPHIC COLUMN
2km
Olivine–basalt group
Graenavatn porphyritic group
Kollur porphyritic group
1km
Reydarfjordur porphyritic basalt
Grjota olivine–basalt group
0
1000
0
-1000
-2000m
573
948
Approximate position of the Reydardfjordur Drill Hole
0 1 2 3 km.
Horizontal and Vertical scale
Map area

Fig. 1 Map and section showing the geology in the vicinity of the IRDP Reydarfjordur drill site.

It is not possible to correlate the major part of the drill-hole succession with the observed up-dip lava sequences exposed on the north and south sides of Reydarfjordur. This was unexpected in view of the regular nature of the exposed geological structure but several features may explain this situation. First, minor intrusions may cut out some key horizons. Second, an unknown amount of down-dip thickening of the lava units may occur—a simple extrapolation of the rate of thickening observed in the sub-aerial section may not be applicable to the lower part of the section. Third, gradual but large-scale lateral changes in the lava stratigraphy may exist.

Aside from difficulties in making specific correlations between the drill hole and exposed, up-dip sections, a striking general result is the paucity of olivine-bearing basalts in the 3-km crustal section, in contrast with a frequency of occurrence of 23% (ref. 2) in the Reydarfjordur succession as a whole. This paucity of olivine-bearing rocks may relate to the early manifestation of the Thingmuli and Breiddalur centres, which erupted over-saturated basaltic lavas and silicic 'differentiates'.

Minor intrusions

Steeply dipping north–south basaltic dykes that are approximately normal to the flows are found at all levels in the exposed section. Dyke density increases notably towards sea level reaching a dilation value of 10%, measured over a 1.6-km transect normal to the swarm, through the drill site. In spite of the obvious drawback in sampling near-vertical rock units with a vertical drill hole, the excellent core recovery allows dykes to be identified in the core on the basis of their chilled contacts, their essentially non-vesicular character, and frequently by their large vertical extent. An example of the multiple dykes which occur is the particularly thick complex of dykes between 528 m and 586 m (units 90.1, 90.2, 90.3, 90.4, 91.2, 92.1, 94.1 and 94.2). There is a broad distribution of intrusive contact angles in the core. Over 40% of these contact angles lie between 55° and 75°. The remainder are rather uniformly distributed over all other angles. In total, 98 minor intrusions constitute 40% of the drill core; however, 11 of these, with vertical extent of greater than 20 m, account for over half of this percentage. The seven minor intrusions in the uppermost 400 m of the drill core comprise five isolated examples and one two-component multiple dyke of 110 m vertical extent (units 10.1 and 17.1, 63 to 173 m). Below 400 m the distribution of minor intrusions is markedly non-uniform. Minor intrusions are concentrated in three intervals each of about 300 m vertical extent separated by comparable intervals in which intrusions are generally much less common (Fig. 2*b*).

Preliminary observations suggest that the minor intrusions are generally less altered than the intervening flows. Thin-section studies indicate that at least some of the dykes in the core are olivine bearing and thus may not be directly related to the lavas of the drill core which are mainly olivine free.

Some of the minor intrusions occurring below 400 m in the drill core are almost certainly gently dipping sheets of a type characteristic of the deeper parts of many Icelandic Tertiary and Quaternary volcanic centres. There is no demonstrable increase in the proportion of minor intrusions as a whole with depth in the drill core (0–500 m, 34%; 500–1,000 m, 42%; 1,000–1,500 m, 45%; 1,500–1,919 m, 39%) or in the proportion of thick, apparently steeply dipping dykes.

Secondary mineralisation

A generally progressive increase of alteration with depth in the section characterises the lavas but is not obvious in the dykes. The topmost flows in the exposed section are unzeolitised. Zeolites appear lower in the exposed section (Fig. 2*a*). In general laumontite is absent in the exposed section, but is found in the Skessa tuff, a silicic ash-flow 100 m above the drill site.

All the flows in the drill core are variably altered, with macroscopic secondary minerals abundantly developed in the brecciated and vesicular flow tops and bottoms. Laumontite is dominant in the upper 1,000 m of the core. A shift from smectite to chlorite dominated assemblages occurs at the 200–300-m depth. Epidote first becomes very common at 1,200 m, where it occurs in both amygdales and veins and in the groundmass of the basalts. From 1,200 m to the bottom of the core at 1,919 m epidote is patchily distributed with alternating rich and deficient sequences. Calcite, various forms of silica and 'celadonite' are common secondary minerals throughout the drill core and various parts of the exposed section. Of particular interest are grossular garnet at dyke contacts at 425 and 1,166 m and anhydrite at 1,055 m.

The alteration pattern of the lavas in the drill core is very similar to that found in other drilled sections in the Tertiary formations of Iceland[7].

Clearly the secondary mineralisation took place at a very much higher temperature than that measured in the hole at present. Based on data from drill holes in the active volcanic zone of Iceland, the lower boundary for the formation of laumontite seems to be about 110 °C and that for epidote about

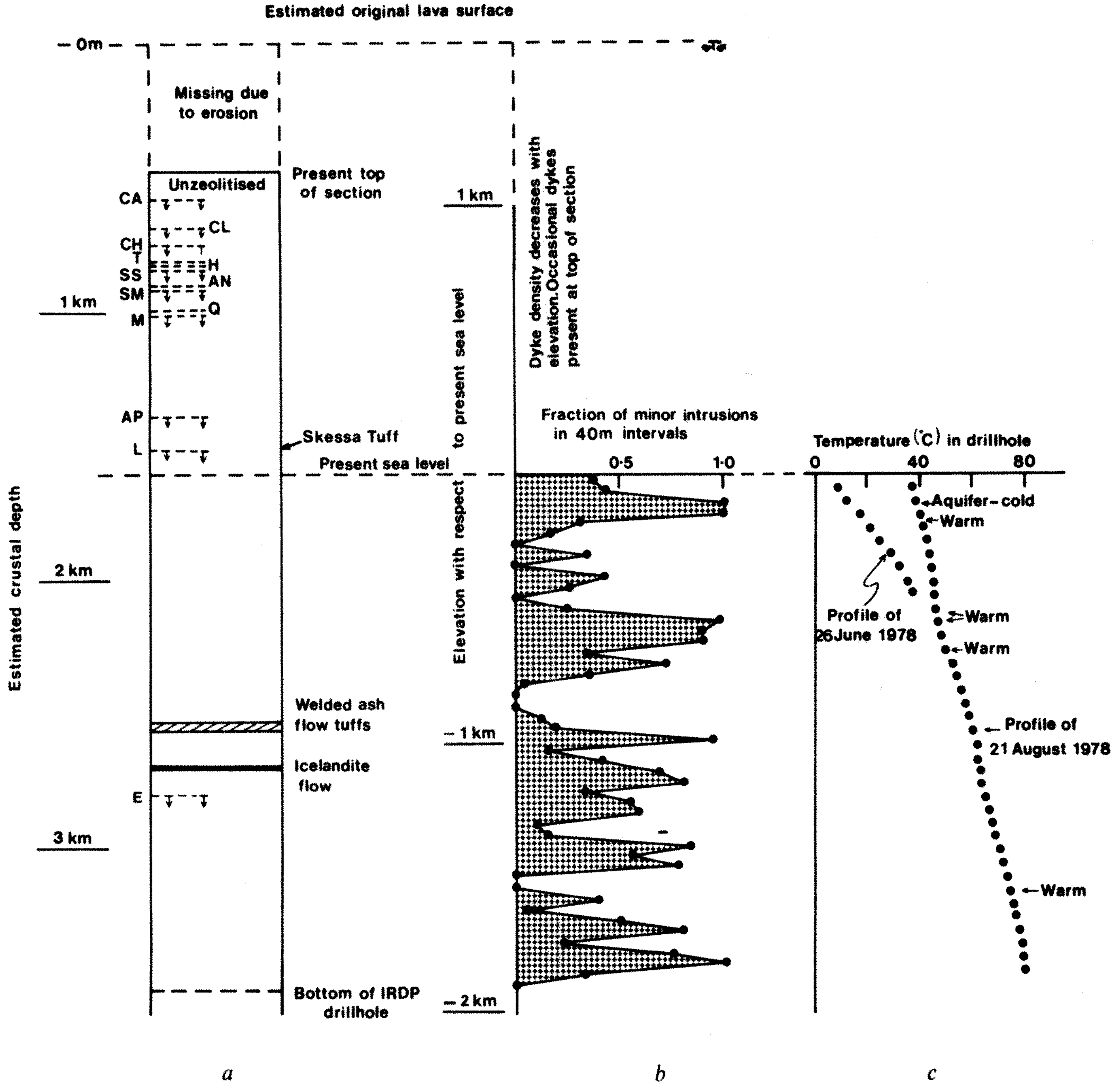

Fig. 2 Profiles through the 3-km Icelandic crustal section. *a*, Marker horizons and highest occurrence of key secondary minerals are indicated by a dashed line and downward pointing arrows. CA, calcite; CL, clay; CH, chabazite; T, thomsonite; H, heulandite; SS, stilbite; AN, analcite; SM, scolecite–mesolite; Q, quartz; M, mordenite; AP, apophyllite; L, laumontite; and E, epidote. *b*, Fraction of minor intrusions in each 40 m depth interval. *c*, Temperature profiles before and after penetration of the series of artesian warm water aquifers between 517 m and 628 m depth.

230 °C (ref. 8). Assuming an original surface 1,500 m above sea level and a linear temperature gradient, the figures suggest the existence of a temperature gradient of close to 85 °C km^{-1} during the formation of the secondary mineralisation.

Magnetic properties

Measurements of initial susceptibility and polarity of natural remanence magnetism (NRM) were obtained for the lower 2 km of the section. Susceptibility values were obtained every 50 cm or less, correction being made for the size and cylindrical shape of the core. Although remanence cleaning is necessary to define the polarity succession reliably, natural remanence polarity measurements may be of some value in locating the section with respect to the known time–magnetic polarity sequence.

The most important feature of the susceptibility measurements is a strong and regular overall decrease with depth for the flows from a mean value of 40×10^{-4} e.m.u. cm^{-3} for the 0–480-m depth interval to 14×10^{-4} e.m.u. cm^{-3} for the 1,440–1,919-m interval. Linear extrapolation of this trend in mean values for the lavas suggests that susceptibility would reach zero value at 2,600 m below sea level or at just over 4 km beneath the original surface of the lava pile. In contrast, no depth trend in minor intrusion susceptibility occurs, with mean values for 480-m depth intervals varying irregularly from 26 to 31×10^{-4} e.m.u. cm^{-3}. Susceptibility typically varies little within minor intrusions, except where fractures or intervals of high permeability occur. In contrast, strong within-unit variation is common for flows, with minima at boundaries and one or two interior maxima. In a general sense, susceptibility variation follows the alteration pattern: the downhole decrease in flow values matches the increase in alteration grade from a laumontite stage to an epidote stage while the minima at flow boundaries correspond to maximum secondary mineral development in these porous regions.

The relatively high average values, and lack of a trend with depth in minor intrusion susceptibility, corresponds with the apparent low alteration state of these units, and may also be indicative of their much younger age. Decomposition of the iron–titanium oxides resulting from zeolite and epidote grade metamorphism is likely to explain reduction of susceptibility in the flows.

Above 1,230 m depth only normal NRM polarity occurs. Reverse NRM polarities, including shallow inclinations, occur mainly within the following depth intervals: 1,445–1,635, 1,690–1,730 and 1,882–1,919 m. Multiple changes of NRM polarity within units have frequently been found in the lower bore-hole section.

At this stage the extended normal polarity zone of the upper half of the core apparently correlates with magnetic anomaly 5 (magnetic epoch 9).

Geothermal measurements

Temperature profiles in the drill hole were obtained weekly, in each case after a 24-h cessation of drilling. Initial water samples were taken from four levels several days after the termination of drilling, but these showed signs of mixing with drilling fluid and the sampling will be repeated. A temperature profile measured when the well was 445 m deep (Fig. 2*c*) showed an approximately linear thermal gradient of about 80 °C km^{-1}. Between 517 m and 628 m a series of prominent artesian aquifers with water at 48 °C were penetrated. These aquifers were tested when the hole was at a depth of 650 m and produced a net flow of 0.8 l s^{-1}. Several smaller aquifers were penetrated at greater depths. Flow of water from the prominent aquifers led to a major change in thermal regime in the hole (Fig. 2*c*); the temperature gradient below these aquifers is much lower.

The site was located within an area of anomalously high surface temperature gradient, and results from the drill hole strongly suggest that this anomalous area is the result of shallow regional geothermal flow of water at moderate temperatures.

Geophysical logging

Extensive geophysical logging of the drill hole was carried out by the Icelandic National Energy Authority with natural γ-ray, neutron–neutron porosity, γ–γ density, caliper, self-potential, 0.4 m and 1.6 m normal resistivity, temperature, differential temperature, sonic velocity and differential magnetic permeability recorded. In the cased central part of the hole (580–1,350 m) self-potential, resistivity and differential permeability could not be recorded. Sonic velocity was recorded from the surface to 1,350 m depth.

Preliminary results show a very pronounced relationship between the physical and lithological parameters. An example is the high resistivity contrast between the massive centres of lava flows and the brecciated or rubbly margins. Dykes and intrusions are also characterised by high resistivity. Relatively few, but prominent, anomalies in the natural γ-ray log are recorded. The sequence of welded ash-flow tuffs between 920 and 951 m depth is characterised by high natural radioactivity and low γ–γ density. Thin breccia layers can be identified in the neutron–neutron porosity log as well as in the γ–γ density log.

Crustal seismology

Two crustal seismic experiments were carried out in association with the drilling project, one by a group from Leningrad and the other by a group from the University of Washington and the Scripps Institution of Oceanography. The results from these experiments are still being interpreted.

Discussion

Although intensive laboratory studies of the 3-km section are continuing, some important points are already evident. Perhaps the most striking feature is the continuously sub-aerial nature of the lavas implying that at least 2 km of subsidence has occurred. No pillowed basalts were found. The absence of marked erosional features in the exposed upper part of the section suggests that the lavas were erupted in an area of low relief at most a few hundred metres above sea level and the implication is that subsidence probably took place concomitant with the volcanism at a rate of at least 1 km Myr^{-1}. Subsidence on this scale is implied in many of the models for crustal growth in Iceland (see ref. 9) and is observed elsewhere where active, volcanic zones occur in areas of oceanic crust[10–12].

The progressive change in the nature of the alteration with depth is also striking. The intensity of metamorphism in the lavas increases gradually, but in detail relatively fresh rocks interfinger with more altered horizons. If the layer 2/layer 3 boundary is fundamentally a metamorphic transition then the present study suggests that such a transition may take place over a distance of the order of 1 km.

The combination of the olivine-bearing nature of some of the minor intrusions, the magnetic properties, and the relative freshness of most of them may imply that they were not feeders for the bulk of the lava succession. The possibility that the dykes are related to the Breiddalur centre[13], 20 km to the south, and that they have been injected laterally northwards in the manner that is presently being observed in the Krafla volcano in north Iceland[14] must be considered.

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Isolation, cloning and sequence analysis of the cDNA for the α-subunit of human chorionic gonadotropin

John C. Fiddes & Howard M. Goodman

Howard Hughes Medical Institute Laboratory, Department of Biochemistry and Biophysics, University of California, San Francisco, California 94143

A 621-base pair fragment of the cDNA for the α-subunit of human chorionic gonadotropin has been isolated by cloning in a plasmid vector, and the complete nucleotide sequence determined. The entire coding region, including the 24-amino acid pre-sequence and most of the untranslated regions, are present in the fragment.

HUMAN chorionic gonadotropin (HCG) is a glycoprotein hormone which is synthesised by the syncytiotrophoblast cells of the placenta. It functions during pregnancy to convey a signal from the placenta to the corpus luteum which results in the maintenance of the corpus luteum and an increased synthesis of steroid hormones (for reviews see refs 1, 2). Certain malignant tumours, not necessarily of placental tissue, also synthesise HCG[3,4]. Detection of HCG by radioimmunoassay is therefore diagnostic of pregnancy or the presence of a tumour[5].

In addition to its medical importance, HCG is an interesting system for studying temporal gene regulation during development. Levels of HCG in the urine can be detected a few days after implantation of the fertilised ovum[6]. Production of the hormone reaches a peak level of about 1 μg ml^{-1} in the plasma at 10–12 weeks and then declines considerably during the second and third trimesters[7]. It is not understood what controls this rise and decline of HCG synthesis during pregnancy nor why such large amounts of the hormone are synthesised towards the end of the first trimester.

The three anterior pituitary hormones, luteinising hormone (LH), follicle-stimulating hormone (FSH) and thyroid-stimulating hormone (TSH), are closely related to HCG in that all four hormones are glycosylated and have a dimeric structure comprising an α-and a β-subunit[1,2,8]. The amino acid sequence of the α-subunit, which is 92 residues long, seems to be identical in all four hormones[9–15], whereas the amino acid sequences of the β-subunits vary[10,16–22]. The β-subunits confer the biological specificity to the hormones[1,2]. Sequence homologies exist between the β-subunits, and to a lesser extent between the β-subunits and the α-subunit, leading to the proposal that all five subunits evolved from a common ancestral gene[23].

The glycoprotein hormones therefore provide an interesting group of structurally related sequences which have been studied extensively in terms of their protein chemistry and biological functions but for which very limited information is available about their mRNA and genomic organisation. This report describes the isolation, by cloning in pBR322, of a 621-base pair cDNA fragment synthesised from first-trimester placental polyadenylated mRNA. The fragment contains the coding sequences for the 92 amino acids of the α-subunit of HCG, the 24 amino acid pre-sequence and most of the 5′- and 3′-untranslated regions.

In vitro translation of placental RNA

Full-term and first-trimester placental mRNA has been analysed by *in vitro* translation and cDNA synthesis to determine whether the higher levels of HCG produced by first-trimester placentae are reflected by higher amounts of cDNA than occur in full-term placentae.

The major protein product synthesised by full-term placentae is the hormone human chorionic somatomammotropin (HCS). Full-term placental polyadenylated mRNA has been analysed previously by wheat-germ translation and immunoprecipitation of the translation products with an antiserum against HCS[24]. The predominant translational product was a protein of molecular weight (MW) 24,000, which contained tryptic peptides in common with HCS[25], and which was precipitated specifically by the antiserum and thus identified as the pre-form of HCS. A similar approach has been used here to analyse first-trimester placental polyadenylated mRNA, which should be enriched for HCG-specific mRNA. The *in vitro* translation products have been compared with those obtained from full-term mRNA, which should have much less HCG-specific mRNA.

DNA was extracted from human placenta obtained either at term (from caesarian sections) or from first-trimester abortions (about 10–12 weeks). The RNA samples were translated in a wheat-germ cell-free translation system with ^{35}S-methionine and the products fractionated on an SDS–15% polyacrylamide gel (Fig. 1). Full-term placental RNA (lane 3) shows the major product of MW 24,000 which has been identified as pre-HCS[24,25]. This product is still observed with first-trimester placental RNA (lane 2) but at a considerably lower level. This is consistent with the observations made by Boime *et al.*[25] and by Chatterjee *et al.*[31], who have also compared the synthesis of HCS by full-term and first-trimester RNA in cell-free translation systems.

The major proteins synthesised *in vitro* by first-trimester RNA (lane 2) have estimated MWs of 13,000 and 16,000 and are only observed in trace quantities with full-term RNA (lane 3). These products are candidates for the pre-forms of the α- and β-subunits of HCG, respectively, as the respective MWs of the

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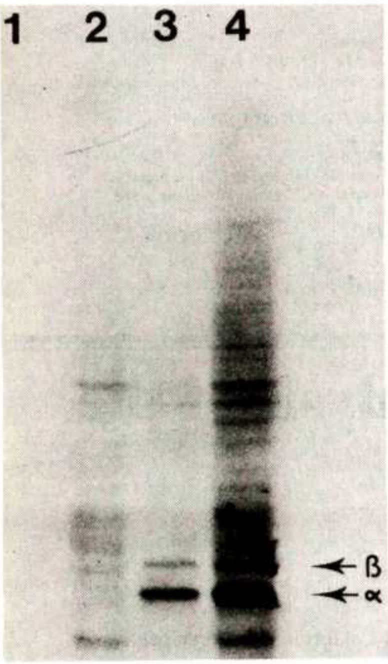

Fig. 1 Wheat-germ translation products of full-term and first-trimester placental mRNA. Human placental tissue was obtained from either caesarian sections or first-trimester abortions. The tissue was washed in cold saline to remove blood, frozen quickly in liquid nitrogen and stored at −70 °C. RNA was prepared by methods similar to those described previously[24,26]. Ten grammes of frozen tissue were added to 100 ml of 5 M guanidine thiocyanate in 50 mM Tris-Cl, *p*H 7.5, 10 mM EDTA and 5% 2-mercaptoethanol and homogenised in a Tissumizer (Tekmar) until all the tissue was dispersed. The solution was centrifuged at 10,000 r.p.m. for 10 min and the supernatant adjusted to 2% Sarkosyl and heated at 65 °C for 2 min. Caesium chloride was then added (0.1 g ml^{-1} of solution) and the resulting solution was layered over 6-ml cushions of half-saturated CsCl in 10 mM EDTA in SW 27 cellulose nitrate tubes. Centrifugation was at 25,000 r.p.m. for 16 h at 20 °C. The RNA pellet was dissolved in 5 mM EDTA, 0.5% Sarkosyl and 5% 2-mercaptoethanol, extracted with phenol and chloroform and precipitated with ethanol. Usually, 0.5–1 mg of RNA were obtained per g of tissue. The RNA was then passed over an oligo(dT)-cellulose column[27] to enrich for the polyadenylated fraction. RNA samples were translated by wheat-germ cell-free extracts[28,29]. Reaction mixtures (40 μl) contained 20 mM HEPES buffer, *p*H 7.6, 2 mM dithiothreitol, 0.1 mM spermidine, 80 mM K acetate, 1 mM Mg acetate, 1 mM ATP, 0.02 mM GTP, 8 mM creatine phosphate, 40 μg ml^{-1} creatine phosphokinase, ~25–50 μCi ^{35}S-methionine (400–900 Ci mmol^{-1}, Amersham), 50 μM each of the other 19 amino acids, 16 μl of wheat-germ extract and 0.5–1.0 μg polyadenylated RNA. Incubation was at 28 °C for 1 h. The products were analysed on an SDS–15% polyacrylamide slab gel[30] and the dried gel autoradiographed. Lane 1, endogenous wheat-germ products; lane 2, first-trimester placental RNA; lane 3, full-time placental RNA. The proposed identifications of the translational products, as discussed in the text, are indicated.

mature forms of these proteins, without glycosylations, are 10,200 and 15,500 (refs 9, 10, 16–18).

The wheat-germ products synthesised in the presence of first-trimester RNA were immunoprecipitated by an antiserum directed against mature HCG and the products fractioned on an SDS–15% polyacrylamide gel (Fig. 2). The translational products generated by first-trimester RNA are shown in lane 4 and the immunoprecipitated products in lane 3. Both the 13,000 and 16,000 MW products seem to precipitate specifically, supporting their identification as pre-forms of the α- and β-subunits of HCG, respectively. In general, however, the immunoprecipitation reactions were inefficient and unreliable. The weak antibody–antigen reaction is presumably related to the fact that the antibody was raised against the mature glycosylated dimeric form of HCG and not against the non-glycosylated pre-forms of the individual subunits which are produced in cell-free translation systems. A small amount of pre-HCS is also precipitated in this reaction (lane 3) but this seems to be the result of a specific reaction between HCS and the formaldehyde-treated *Staphylococcus aureus* cells used to collect the antigen–antibody complex, because it also precipitates in the absence of antibody but in the presence of the *S. aureus* cells.

The data presented here are consistent with other results. It is reasonable to expect a greater quantity of α- than β-specific mRNA as there is an excess amount of free α-subunit over free β-subunit in plasma[33]. Also, Landefeld *et al.*[34] have shown that the major translational product from first-trimester RNA has tryptic peptides in common with the native α-subunit. Daniels-McQueen *et al.*[35] and Chatterjee *et al.*[31,36] have observed lower levels of β-subunit synthesis than α-subunit synthesis in cell-free systems and have demonstrated a greater amount of synthesis of these subunits with first-trimester than with full-term RNA.

Restriction enzyme analysis of placental cDNA

Discrete restriction endonuclease fragments can be generated from heterogeneous cDNA if that cDNA contains a reasonably enriched species. This approach has been used to clone fragments of human growth hormone, HCS, rat growth hormone and rat insulin cDNAs[37–40]. The differences in translational activities observed between first-trimester and full-term human placental RNA should therefore also be observed in the restriction enzyme fragments generated from cDNA synthesised from these two sources of mRNA. Comparison of the two cDNA fragment patterns should allow identification of fragments in first-trimester cDNA which probably correspond to either α- or β-HCG and thus facilitate cloning of these cDNA species.

Double-stranded cDNA was synthesised from polyadenylated RNA obtained from both first-trimester and full-term placentae and was restricted with either *Alu*I or *Hpa*II. The products were fractionated by electrophoresis on a 5% polyacrylamide gel (Fig. 3). With both enzymes a specific band pattern is observed which varies with the RNA used to generate the cDNA. The 340-base pair fragment obtained when full-term cDNA is digested with *Alu*I (lane 1) is known from sequence analysis to be present in HCS cDNA[38] and is only observed at a very low level in first-trimester cDNA (lane 2). In contrast, *Alu*I digestion of first-trimester cDNA (lane 2) generates fragments

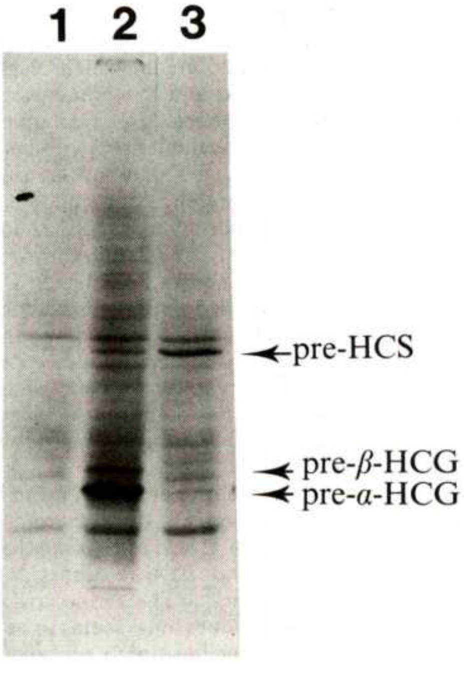

Fig. 2 Immunoprecipitation of wheat-germ products by HCG antiserum. The wheat-germ translation products generated by first-term placental RNA (Fig. 1) were immunoprecipitated by an antiserum directed against native HCG and the antigen–antibody complex collected by formaldehyde-treated *S. aureus* cells[29,32]. The products were fractionated on an SDS–15% polyacrylamide gel[30] and visualised by autoradiography of the dried gel. Lane 1, immunoprecipitation of endogeneous wheat-germ products; lane 2, endogenous wheat-germ translation products; lane 3, immunoprecipitation of wheat-germ translation products directed by first-trimester placental polyadenylated RNA; lane 4, wheat-germ products of first-trimester placental polyadenylated RNA. The proposed identifications of the pre-forms of the α- and β-subunits of HCG are indicated.

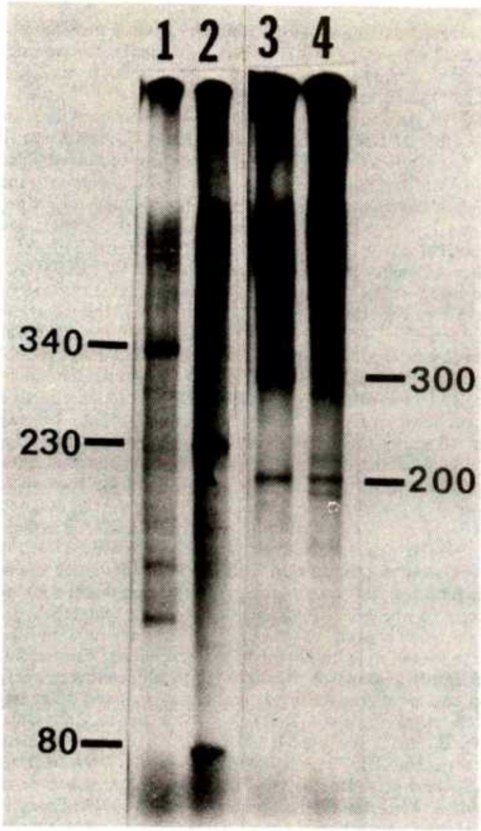

Fig. 3 Restriction enzyme analysis of double-stranded cDNA made from polyadenylated RNA extracted from both full-term and first-trimester human placentae. The methods used for synthesis of double-stranded cDNA were similar to those described previously[37,39,41]. For preparative syntheses, ~20 μg polyadenylated RNA were incubated in 200 μl reactions containing 50 mM Tris-Cl, *p*H 8.3, 10 mM $MgCl_2$, 20 mM KCl, 10 mM 2-mercaptoethanol, 500 μM each of dATP, dCTP, dGTP and dTTP (one [α-^{32}P]-nucleoside triphosphate at specific activity 500 c.p.m. ^{32}P per pmol), 20 ng μl^{-1} oligo$(dT)_{12-18}$ and 200 units reverse transcriptase. Incubation was at 42 °C for 30 min and was stopped by the addition of EDTA to 10 mM. After extraction with phenol and chloroform and precipitation by ethanol, the material was fractionated on a 25 × 0.25 cm column of Sephadex G-50 in 1 mM Tris-Cl, *p*H 7.4, and 0.01 mM EDTA. The leading peak was collected (~200 μl) and adjusted to 0.3 M NaOH and 10 mM EDTA and incubated overnight at room temperature. Following neutralisation with sodium acetate, *p*H 5.5, and ethanol precipitation, the second strand was synthesised in a 50-μl reaction containing the same buffer as described above, 500 μM each of dATP, dGTP and dTTP and 100 μM dCTP (10,000 c.p.m. ^{32}P per pmol) (considerably higher specific activities were used for analytical reactions) and 50 units reverse transcriptase. Incubation was at 42 °C for 90 min. For subsequent restriction enzyme analysis the reaction was stopped by heating to 65 °C for 10 min and the appropriate restriction enzymes added directly. Alternatively, the reaction products were fractionated as described above on a Sephadex G-50 column and the peak evaporated to dryness before proceeding to treatment with S_1 nuclease (see Fig. 4 legend). Lane 1, *Alu*I digest of full-term placental cDNA; lane 2, *ALu*I digest of first-trimester placental cDNA; lane 3, *Hpa*II digest of full-term placental cDNA; lane 4, *Hpa*II digest of first-trimester placental cDNA. Sizes are in base pairs.

of 230 and 80 base pairs that are not observed in full-term cDNA. The enzyme *Hpa*II also generates different fragments from full-term and first-trimester cDNAs (lanes 3, 4). It is reasonable to assume that the fragments specific to first-trimester cDNA correspond to either α- or β-HCG cDNA.

Cloning of the cDNA for the α-subunit of HCG

The comparisons of the wheat-germ translation products and the cDNAs synthesised from full-term and first-trimester placental polyadenylated mRNAs suggest that it should be possible to clone the HCG cDNA species from first-trimester material without any further enrichment procedures.

Double-stranded cDNA was synthesised by reverse transcriptase from 20 μg of first-trimester polyadenylated mRNA[37,39,41] (Figs 3, 4). Yields of about 5% and 25% were obtained on the first- and second-strand syntheses, respectively. An aliquot of the double-stranded cDNA was electrophoresed on a 5% polyacrylamide gel and the products visualised by autoradiography (Fig. 4, lane 2). A prominent band corresponding to a chain length of about 700 base pairs can be seen above the heterogeneous cDNA background. This species was also observed, but with the expected slower mobility, in the single-stranded cDNA (Fig. 4, lane 1). Presumably, this species corresponds to a full-length cDNA for either the α- or β-subunit of HCG, as 700 base pairs has sufficient coding capacity for either subunit plus untranslated regions, but not for the two together. Cleavage of the double-stranded cDNA with the enzyme *Hin*dIII generates a new band (about 630 base pairs) plus a residual low level of the 700-base pair band (Fig. 4, lane 4). As this was observed reproducibly it is not believed to be the result of partial digestion. Instead, it is interpreted to mean that the initial undigested 700-base pair band contains two cDNA species, presumably corresponding to the α- and β-subunits of HCG, and that the major species of cDNA contains a *Hin*dIII site about 50 base pairs from one end.

The full-length cDNA in the size range 600–750 pairs was then cloned into the unique *Hin*dIII site of pBR322 using synthetic *Hin*dIII linkers as described in Fig. 4 legend. Small plasmid DNA preparations were made from the 22 recombinants obtained in the transformation and analysed by restriction enzyme digestion. The enzymes *Pst*I and *Xba*I were used because first-trimester cDNA cleaved with these enzymes produces specific fragments, about 230 base pairs long in the case of *Xba*I and about 380 and 170 base pairs long in the case of *Pst*I (Fig. 4, lanes 7, 8). These fragments are presumably diagnostic of HCG cDNA and can be readily detected, as the plasmid pBR322 contains a single *Pst*I cleavage site and no *Xba*I cleavage site[44]. One recombinant was obtained; it had a *Hin*dIII insert about 620 base pairs long which when digested with *Xba*I gave fragments of 400 and 220 base pairs and when digested with *Pst*I gave fragments 370, 170 and 80 base pairs long. In addition, this 620-base pair *Hin*dIII fragment contains *Alu*I and *Hpa*II fragments consistent with those observed in first-trimester cDNA (Fig. 3). This recombinant cDNA clone was thus considered a candidate for the α-subunit of HCG because it corresponds to the major cDNA species. No candidates for β-HCG cDNA have been obtained.

DNA sequence analysis of α-HCG cDNA

DNA sequencing was used to show that the *Hin*dIII fragment codes for the α-subunit of HCG and is 621 bases long. Initial identification of the recombinant, designated α-HCG/pBR322, was by a modification of the sequencing technique described by Maat and Smith[45] for sequencing 5′-terminally labelled DNA fragments by the chain termination method (A. J. H. Smith, personal communication). The end-labelled DNA fragments were incubated with DNA polymerase I (Klenow modification) and a single dideoxynucleoside triphosphate, and the products were loaded directly onto a DNA sequencing gel (see Fig. 5 legend). This method results in the introduction of the chain terminators into the double-stranded DNA, presumably at the sites of nicks in the DNA caused by the contaminating DNase in DNA polymerase I preparations. This was found to be a particularly rapid method for analysing potential recombinants, but difficulties were encountered in obtaining accurate sequences where there were stretches of a single nucleotide.

The complete nucleotide sequence of the recombinant was obtained following transfer of the 621-base pair *Hin*dIII fragment to the single-stranded DNA phage vector M13 mp5. Standard chain termination methods were used[46,48] and, where possible, sequences were obtained on both strands, but in all cases the results were confirmed by additional primings. The complete 621-base pair sequence is presented in Fig. 5.

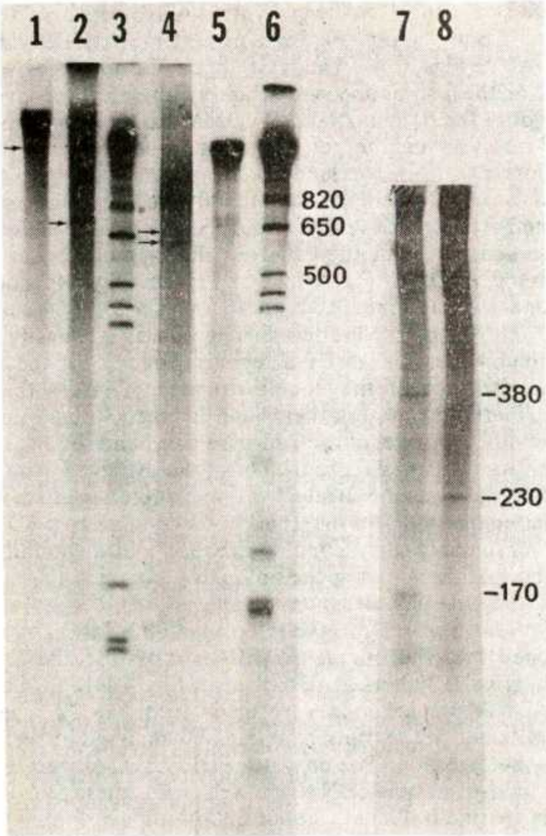

Fig. 4 Synthesis and cloning of first-trimester cDNA. The methods used for cloning the cDNA using *Hin*dIII linkers were similar to those described elsewhere[37,39]. Synthesis of double-stranded cDNA from 20 μg polyadenylated RNA was as described in Fig. 3 legend. After fractionation by Sephadex G-50, the double-stranded cDNA was incubated with 5 units of S_1 nuclease in 300 mM NaCl, 30 mM Na acetate, *p*H 4.5, and 3 mM $ZnCl_2$ at 42 °C for 5 min (refs 37, 41). The reaction was stopped by the addition of EDTA to 10 mM and neutralised with Tris base. Following extraction with phenol and chloroform, the products were fractionated over a Sephadex G-50 column (Fig. 3) and the peak fraction evaporated to dryness. The DNA was then incubated in a 25-μl reaction with 50 mM Tris-Cl, *p*H 7.5, 10 mM $MgCl_2$, 10 mM 2-mercaptoethanol, 25 μM each of dATP, dCTP, dGTP and dTTP and 1 unit of DNA polymerase I (Klenow fragment, Boehringer) at 12 °C for 15 min. The reaction was adjusted to 50 μl in the same buffer with 1 mM ATP and 12 μg kinase-treated [5'-^{32}P]-labelled *Hin*dIII decanucleotide linkers (2×10^6 c.p.m. total) and incubated with 0.3 units T4 DNA ligase and 2.5 units T4 RNA ligase (Biolabs) at 12 °C overnight. After heating the reaction to 65 °C for 10 min, 50 units *Hin*dIII were added directly and the reaction was incubated at 37 °C for 2 h. Aliquots of the reaction were assayed at all stages by electrophoresis on 5% polyacrylamide gels and autoradiography of the dried gels. Following extraction with phenol and chloroform, the cDNA, with cohesive *Hin*dIII ends, was fractionated on a preparative 5% polyacrylamide gel and fragments in the size range 600–750 base pairs recovered by electrophoretic elution. The cDNA was then ligated to 100 ng *Hin*dIII-cleaved, alkaline phosphatase-treated pBR322 (refs 37, 39, 42) and used to transform *E. coli* χ1776 as described elsewhere[43]. Transformation and subsequent growth were carried out in a P3 facility in compliance with the NIH Guidelines for Recombinant DNA Research. Twenty-two colonies were obtained which were resistant to 20 μg ml^{-1} ampicillin and sensitive to 20 μg ml^{-1} tetracycline. The recombinants contained *Hin*dIII inserts of the appropriate size and were analysed by restriction enzyme digestion, in particular with *Pst*I and *Xba*I (see text). Lane 1, full-length single-strand cDNA; lane 2, full-length double-strand cDNA; lane 3, *Hpa*II digest of M13 DNA; lane 4, double-stranded cDNA digested with *Hin*dIII; lane 5, double-stranded cDNA digested with nuclease S_1; lane 6, as lane 3; lane 7, *Pst*I digestion of double-stranded cDNA; lane 8, *Xba*I digestion of double-stranded cDNA. Lanes 1–4, 5–6 and 7, 8 are from separate gels. Autoradiographs were made from dried 5% polyacrylamide gels. Sizes are in base pairs.

```
                                                    -24             -20                                     -10
                                                    met asp tyr tyr arg lys tyr ala ala ile phe leu val thr leu ser val
CAGTAACCGGCCCTGAACACATCCTGCAAAAAGCCCAGAGAAAGGAGCGCC ATG GAT TAC TAC AGA AAA TAT GCA GCT ATC TTT CTG GTC ACA TTG TCG GTG
1                            30               HhaI          60                  AluI                90

                            1                                       10                                      20
phe leu his val leu his ser ala pro asp val gln asp cys pro glu cys thr leu gln glu asn pro phe phe ser gln pro gly ala
TTT CTG CAT GTT CTC CAT TCC GCT CCT GAT GTG CAG GAT TGC CCA GAA TGC ACG CTA CAG GAA AAC CCA TTC TTC TCC CAG CCG GGT GCC
                     120                                    150                                    180     HpaII

                        30                                      40                                      50
pro ile leu gln cys met gly cys cys phe ser arg ala tyr pro thr pro leu arg ser lys lys thr met leu val gln lys asn val
CCA ATA CTT CAG TGC ATG GGC TGC TGC TTC TCT AGA GCA TAT CCC ACT CCA CTA AGG TCC AAG AAG ACG ATG TTG GTC CAA AAG AAC GTC
                     210                  XbaI                  240                                    270

                        60                                      70                                      80
thr ser glu ser thr cys cys val ala lys ser tyr asn arg val thr val met gly gly phe lys val glu asn his thr ala cys his
ACC TCA GAG TCC ACT TGC TGT GTA GCT AAA TCA TAT AAC AGG GTC ACA GTA ATG GGG GGT TTC AAA GTG GAG AAC CAC ACG GCG TGC CAC
         HinfI       300     AluI                           330                                    360

                        90      92
cys ser thr cys tyr tyr his lys ser OC
TGC AGT ACT TGT TAT TAT CAC AAA TCT TAA ATGTTTTACCAAGTGCTGTCTTGATGACTGCTGATTTTCTGGAATGGAAAATTAAGTTGTTTAGTGTTTATGGCTTTGTGA
PstI                 390                            420                       450                            480

GATAAAACTCTCCTTTTCCTTACCATACCACTTTGACACGCTTCAAGGATATACTGCAGCTTTACTGCCTTCCTCCTTATCCTACAGTACAATCAGCAGTCTAGTTCTTTTCATTTGGAA
                          510                          PstI AluI                 570                          600

TGAATACAGCATTAAGCTT
              HindIII
```

Fig. 5 Nucleotide sequence of the 621-nucleotide-long cDNA fragment coding for the α-subunit of HCG. The amino acid sequence of the 92-residue-long mature protein is shown as well as the 24-residue pre-sequence deduced from the cDNA sequence. Nucleotides are numbered below the sequence and amino acids above the sequence. The restriction endonuclease sites used in the sequence analysis are indicated. DNA from α-HCG/pBR322 was cleaved at the unique *Xba*I site, labelled with [γ-^{32}P]ATP by polynucleotide kinase and then digested with *Hin*dIII. The 400-base pair *Hin*dIII–*Xba*I fragment was isolated and divided into four separate 5-μl reactions each containing 6 mM Tris-Cl, *p*H 7.4, 6 mM $MgCl_2$, 1 mM dithiothreitol, 0.2 mM of a single dideoxynucleoside triphosphate and 1 unit DNA polymerase I (Klenow modification) and incubated at 37 °C for 1 h (ref. 45 and A. J. H. Smith, personal communication). The products were analysed directly on thin sequencing gels[46]. After the 621-base pair fragment had been shown by this method to code for α-HCG, it was transferred to the unique *Hin*dIII site of the single-stranded filamentous DNA phage vector M13 mp5. This phage is a derivative of M13 mp2 (ref. 47); its transformation and growth (J. Messing, unpublished results) were done in a P3 facility in accordance with the NIH Guidelines for Recombinant DNA Research. Initially, the host strain 71/18 was used but has lately been replaced by JM101 (J. Messing, unpublished results). Both the coding and non-coding strands of the cDNA were inserted separately into the viral strand of the phage DNA and were used as templates for DNA sequencing reactions by the chain termination method[48]. Restriction enzyme fragments for use as primers were obtained from α-HCG/pBR322.

The finding that the α- and β-subunits of the glycoprotein hormones are synthesised from separate polyadenylated mRNA species agrees with the deduction made by Daniels-McQueen *et al.*[35], who observed that the α- and β-translational activities for HCG sedimented at different positions in sucrose gradients.

The amino acid sequence for the 92 codons of the mature form of α-HCG as determined previously[9,10] corresponds to that predicted from the DNA sequence (Fig. 5) with the exception of codon 15. Morgan *et al.*[10] identified this position as aspartic acid whereas Bellisario *et al.*[9] did not distinguish between aspartic acid or asparagine. DNA sequence analysis shows that the correct amino acid is asparagine. This assignment agrees with the sequences reported for the α-subunits of human LH and FSH[11,12,14] which are probably identical to α-HCG. The sequences reported for the α-subunits of HCG[9,10] and human FSH[14] have the sequence Cys–Ser at positions 84 and 85, whereas this order is reserved in the reported sequence for human α-LH[11]. The DNA sequence analysis of α-HCG cDNA supports the former identification of this dipeptide. N-terminal heterogeneity has been reported for the α-subunits of human glycoprotein hormones involving the first three amino acids and was assumed to be the result of proteolytic cleavage. The identification of the coding sequence for this region supports this assumption.

A pre-sequence of 24 amino acids can be predicted from the cDNA sequence (Fig. 5) and has the same characteristic hydrophobic stretches observed in other pre-sequences (see ref. 49). This sequence agrees with the preliminary results of Boime *et al.*[50], who have identified a pre-sequence of 24 amino acids for the α-subunit of HCG and have located the positions of the Ala, Phe and Leu residues.

The coding region ends with the termination codon UAA and is followed by 220 bases to the *Hin*dIII site. This restriction enzyme site is not the result of a linker ligation but corresponds to the natural *Hin*dIII site observed in the cDNA. Approximately 50 bases of the 3′-terminal region of the cDNA are thus absent from the clone, including the sequence AAUAAA which is characteristic of all polyadenylated eukaryotic mRNA species[51]. Fifty bases of the 5′-untranslated region are present in the clone before the *Hin*dIII linker molecule. The sequence AAGGAG located at positions −10 to −5 before the initiator AUG shows perfect complementarity with the 3′-end of *Escherichia coli* 16S rRNA and is thus similar to a prokaryotic ribosome binding site[52].

The codon usage observed with the α-subunit of HCG, including the pre-sequence, is shown in Fig. 6. Of the 10 serine codons, nine are of the form UCN and only one is AGPy; all four arginine codons are of the form AGPu and none are CGN.

The similarity in amino acid sequence among all four α-subunits for human CG, LH, FSH and TSH suggests that they are coded for by a single gene. To test this, the 621-base pair fragment was labelled with ^{32}P and used as a probe to hybridise to endonuclease-digested human placental DNA by the method of Southern[53] (Fig. 7). With three enzymes, *Eco*RI (lane 1), *Bgl*II (lane 5) and *Pvu*II (lane 6), single hybridising bands are observed. The *Eco*RI fragment is about 6.5 kilobases long. These results suggest the existence of either a single gene or multiple copies arranged in tandem within the 6.5-kilobase fragment.

2/UUU/phe	2/UCU/ser	5/UAU/tyr	2/UGU/cys
4/UUC/phe	4/UCC/ser	2/UAC/tyr	8/UGC/cys
0/UUA/leu	2/UCA/ser	1/UAA/OC	0/UGA/OP
2/UUG/leu	1/UCG/ser	0/UAG/AM	0/UGG/trp
1/CUU/leu	1/CCU/pro	2/CAU/his	0/CGU/arg
1/CUC/leu	1/CCC/pro	3/CAC/his	0/CGC/arg
2/CUA/leu	4/CCA/pro	1/CAA/gln	0/CGA/arg
2/CUG/leu	1/CCG/pro	4/CAG/gln	0/CGG/arg
0/AUU/ile	3/ACU/thr	0/AAU/asn	1/AGU/ser
1/AUC/ile	1/ACC/thr	4/AAC/asn	0/AGC/ser
1/AUA/ile	2/ACA/thr	4/AAA/lys	2/AGA/arg
4/AUG/met	3/ACG/thr	3/AAG/lys	2/AGG/arg
1/GUU/val	3/GCU/ala	3/GAU/asp	2/GGU/gly
4/GUC/val	1/GCC/ala	0/GAC/asp	1/GGC/gly
2/GUA/val	2/GCA/ala	2/GAA/glu	0/GGA/gly
3/GUG/val	1/GCG/ala	2/GAG/glu	1/GGG/gly

Fig. 6 Codon usage of α-HCG mRNA.

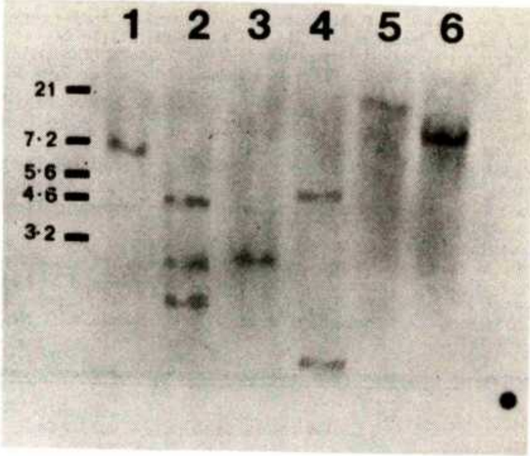

Fig. 7 Autoradiograph of a filter hybridisation between restriction endonuclease-digested human placental DNA, fractionated on a 1% agarose gel, and ^{32}P-labelled, cloned α-HCG cDNA. The filter hybridisation method of Southern[53] was used as described previously[54]. Lane 1, *Eco*RI; lane 2, *Xba*I; lane 3, *Pst*I; lane 4, *Hin*dIII; lane 5, *Bgl*II; lane 6, *Pvu*II. The positions and sizes in kilobase pairs of the fragments produced by an *Eco*RI digest of wild-type bacteriophage λ DNA are shown.

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Note added in proof: A more accurate estimate of the size of the hybridising *Eco*RI fragment is 5.0 kilobases.

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letters

Diffusion of the highest energy cosmic rays from Virgo

J. Wdowczyk

Institute of Nuclear Research, Lodz, Poland

A. W. Wolfendale

Department of Physics, University of Durham, UK

The origin of the predominant particle component of cosmic rays is still undecided although at 'low' energies ($E \sim 10^9$–10^{10} eV) γ-ray data provide circumstantial evidence in favour of an origin in galactic sources[1]. The galactic magnetic field deflects trajectories, at least until about 10^{18} eV, and almost completely destroys any relationship between the directions from which cosmic rays arrive and the directions to their sources, making the elucidation of origins a problem. Above 10^{18} eV, if the primary cosmic rays are mainly protons, as would be expected and as is claimed[3], then the absence of large anisotropies associated with the galactic plane strongly suggests that the particles are from extragalactic sources. If they are extragalactic and their sources are distributed widely in the Universe then, because of interactions between the protons and the photons of the 2.7 K relict radiation, the observed spectrum should steepen considerably above several times 10^{19} eV. However, the opposite appears to be happening: the measured spectrum flattens above about 10^{19} eV (refs 4–6) (see Fig. 2). Several suggestions have been made to account for this paradox, in each case the idea being to generate in extragalactic space a production spectrum which is so 'flat' that even after energy losses are taken into account, the resulting spectrum at the Earth has the characteristic observed flattening (for example ref. 7). Here we put forward an alternative model where the observed spectral shape arises in a rather natural way, being due to propagation characteristics in the local supercluster, the bulk of the very energetic particles having originated in its central region.

Many studies have been made of the diffusion of cosmic rays within the Galaxy but little attention has been given to the possibility of propagation in extragalactic space being by diffusive rather than rectilinear motion. This is due to lack of knowledge of the topography of the magnetic field in space. Recent measurements of X-ray emission from clusters of galaxies (some of which seems to be due to thermal bremsstrahlung from hot intergalactic gas) make us believe, however, that significant magnetic fields exist. For example, if some measure of equipartition exists between field energy ($B^2/8\pi$) and kinetic energy of gas clouds ($\rho v^2/2$) then the likely gas densities of $\approx 10^{-4}$ atoms cm^{-3} in clusters indicate fields of $\approx 10^{-8}$ G.

A necessary parameter for particle diffusion is the cell length over which the field can be regarded as uniform—rough arguments suggest that a dimension of the order of a few galactic diameters (say 0.1 Mpc) might be reasonable. These arguments are: (1) A likely source of at least some of the cluster field is clouds of gas containing cosmic rays which are forced out of the galaxy when supernovae explode. Such clouds, with closed field loops, would presumably have dimensions of a few kpc on emergence from the galaxy and would expand at greater distances. (2) Within the galaxy the cell length seems to be about one hundredth of the mean linear dimension of the galaxy. One hundredth of the characteristic dimension of the supercluster would correspond to a few hundred kpc. There is, of course, no certainty about the magnitude of the cell length adopted, but it does seem as though a value within a factor 10 either way of 100 kpc is likely.

The Larmor radius of a proton of energy E_{20} (in units of 10^{20} eV) in a field of 10^{-8} G is $E_{20}.10$ Mpc, so diffusion might be expected to be very important for particles from all extragalactic sources at energies below $\simeq 10^{18}$ eV and for particles from sources within some tens of Mpc with energy below 10^{20} eV or so. Above a few times 10^{21} eV there will be rectilinear propagation from sources within tens of Mpc.

The attenuation length for protons on the microwave background is well known. This length, λ, equals the distance to the centre of Virgo (19 Mpc) at $E_0 \simeq 3\times10^{20}$ eV. The next 'large' cluster, Pegasus I, is at 65 Mpc, for which $E_0 \simeq 1.3\times10^{20}$ eV. If the sources of particles of energy $\approx 10^{20}$ eV are only present in cluster centres, then these particles will only arrive from Virgo, particles from further afield having had insufficient time to travel to Earth. It is this feature of depressing cosmic rays from sources beyond Virgo that distinguishes the present model from previous ones (for example, the supercluster enhancement models of Strong *et al.*[8] and Stecker[9,10]).

We now consider a model in which particles diffuse from the centre of Virgo and are removed after a mean time T determined by the Hubble time or the attenuation time (λ/c), whichever is the shorter.

Calculations have been made for a variety of forms of the dependence of the diffusion coefficient on energy $D(E)$. General arguments dictate that D should be a function of E but the dependence is not known. For galactic cosmic rays the change of slope of the spectrum at $\sim 3\times10^{15}$ eV is often interpreted as due to a change of the form of $D(E)$ from $D(E)=$

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constant below 3×10^{15} eV to $D(E)\not\propto E^{0.5}$ above. The latter is our preferred choice for the supercluster.

The flux of cosmic rays at a distance x from a continuously emitting source can be shown to be

$$n(x)=\frac{qcT}{2\pi^2x^3}w\phi(\sqrt{2w}),$$

where $w=x^2/4DT$ and

$$\phi(y)=\frac{1}{\sqrt{2\pi}}\int_y^\infty \exp-\frac{s^2}{2}\,ds \tag{1}$$

where q is the rate of emission of particles from the source. It is assumed that D is only a slowly varying function of E. The expression is valid only at energies significantly below E_0 where $D(E_0)=cx/3$; at significantly higher energies straight line propagation occurs and $n(x)=q/4\pi x^2$ (the flux being collimated from the source). For very low energies, where $\phi(y)$ is given by the recurrence formula,

$$n(x)\rightarrow\frac{cq}{8\pi^{5/2}}\frac{1}{x^2D}\sqrt{DT}\exp-\frac{x^2}{4DT} \tag{2}$$

Figure 1 shows $n(x)$ for two forms of $D(E)$. The curves are normalised at 10^{19} eV. Above 10^{20} eV there is increasing inaccuracy as straight line propagation is approached and our estimate is only approximate. The forms chosen for $D(E)$ were $D(E)\propto E^{1/2}$ and $D(E)\propto E$—the galactic situation seems to require the former and we regard this as the preferred expectation for supercluster propagation, at least above 10^{18} eV.

Figure 2 shows the prediction for $D(E)\propto E^{1/2}$ normalised to the experimental results at 10^{19} eV. The required production spectrum is

$$q(E)=7.4\times10^{24}\left(\frac{E(\mathrm{GeV})}{10^{10}}\right)^{-2.44}\ \mathrm{s^{-1}\,GeV^{-1}} \tag{3}$$

and the diffusion coefficient is

$$D(E)=5\times10^{33}\left(\frac{E(\mathrm{GeV})}{10^{10}}\right)^{1/2}\ \mathrm{cm^2\,s^{-1}} \tag{4}$$

There is quite good agreement between prediction and observation, at least above about 5×10^{17} eV (in this model, particles of lower energy would come from galactic sources).

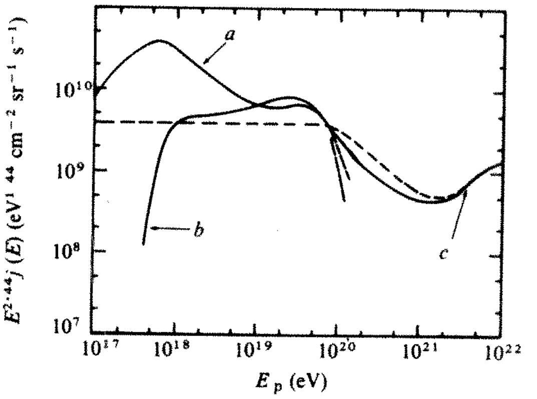

Fig. 1 Spectra of protons expected from the centre of Virgo. Predictions for alternative choices of $D(E)$ are indicated. Chain lines above 10^{20} eV are predictions for the diffusion model in this region; there must be a transition to the situation for straight line propagation (c), which must be reached above about 2×10^{21} eV, and the full line represents an approximate estimate. If there were no extragalactic magnetic field (--- $B_{\mathrm{EG}}=0$) the contribution from Virgo alone would follow the dashed line indicated. The drop above 10^{20} eV is due to interactions with the 2.7 K radiation. An energy-independent $D(E)$ would not give the required upturn in the spectrum. a, $D=5\times10^{33}\sqrt{E(\mathrm{GeV})/10^{10}}\ \mathrm{cm^2\,s^{-1}}$. b, $D=5\times10^{33}(E(\mathrm{GeV})/10^{10})\ \mathrm{cm^2\,s^{-1}}$.

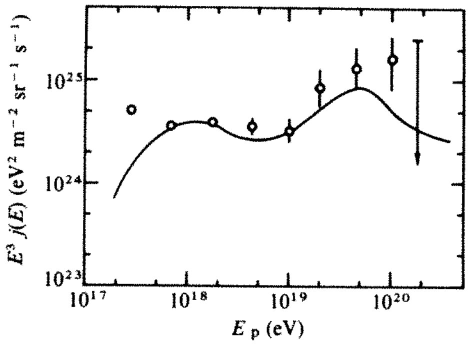

Fig. 2 Spectra of extragalactic protons (from Virgo) predicted using the form of $D(E)\propto E^{1/2}$ given in Fig. 1. The experimental intensities come from a summary of all the experimental measurements[4–6].

The numerical values for the parameters can be examined to see if they are reasonable. $D(E)$ gives $\lambda\simeq150$ kpc at $E=10^{19}$ eV and this is of the expected order of magnitude. The production spectrum, however, gives a high output; extrapolating $q(E)$ back to 1 GeV gives a total rate of energy production of $\sim7\times10^{46}\ \mathrm{erg\,s^{-1}}$, compared with $\sim6\times10^{40}\ \mathrm{erg\,s^{-1}}$ for the Galaxy. This ratio of 10^6 is much bigger than the ratio of 500 or so which pertains at radio wavelengths[11]. A further difference is that the spectral slope ($\gamma=2.44$) is less than that associated with galactic particles below 10^{15} eV. However, a number of points can be made in support of the argument: (1) The exponent of the production spectrum for galactic particles is probably significantly smaller than the measured value of 2.6 (and nearer 'our' 2.44) because of the likelihood of the cosmic ray lifetime falling with increasing energy. (2) Other galaxies near Virgo may also be contributing. More important, with the likelihood of a different acceleration mode for M87, the exponent could be different at very low energies and the total energy produced consequently much smaller.

An indication that the yield of particles from the M87 region as a whole is high is provided by observations of the diffuse X-ray flux from this part of the supercluster. Forman *et al.*[12] quote an emissivity of about $10^{43}\ \mathrm{erg\,s^{-1}}$ for this diffuse component, to be compared with our own estimate of $10^{38}\ \mathrm{erg\,s^{-1}}$ for the Galaxy, that is a ratio 10^5, a result only a factor 10 different from that required on our model for cosmic rays.

Finally some remarks can be made about the expected anisotropy. The magnitude of the anisotropy for particles diffusing from a source in the x direction is $\delta=(3D(E)/CN(x))\times dN(x)/dx$ and this has been evaluated for energies above 10^{18} eV where, on the present model, the particles are largely extragalactic. The values predicted are $\delta=1\%$, 4% and 20% for primary energies of 10^{18}, 10^{19} and 10^{20} eV, respectively. Measured anisotropies to date are imprecise, and firm evidence for finite anisotropies is absent above 10^{18} eV; however, there seems to be a trend in phases with increasing energy[13], not inconsistent with an approach to the Virgo direction above 10^{20} eV. The proof of the present model will clearly rest on the observation of such a collimation of arrival directions at these ultra-high energies.

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An evolution free test for non-zero cosmological constant

Charles Alcock

The Institute for Advanced Study, Princeton, New Jersey 08450

Bohdan Paczyński*

Department of Astronomy, University of California at Berkeley, Berkeley, California 94720 and Princeton University Observatory, Princeton, New Jersey 08540

The cosmological constant has recently been questioned because of difficulties in fitting the standard $\Lambda = 0$ cosmological models to observational data[1,2]. We propose here a cosmological test that is a sensitive estimator of Λ. This test is unusual in that it involves no correction for evolutionary effects. We present here the idealised conception of the method, and hint at the statistical problem that its realisation entails.

Consider a collection of test objects emitting radiation containing spectral lines (so that redshifts may be determined), which are distributed on the surface of a sphere. (Any spherically symmetric, bounded distribution will do; this idealisation is for convenience only.) Let the sphere expand with the local Hubble flow. When observed at a great distance the redshifts of these objects will be confined to some narrow interval $(z - \Delta z, z + \Delta z)$, and their distribution on the sky will be confined in some circle of angular radius $\Delta\theta$. Our task is to evaluate $\Delta z/(z\Delta\theta)$.

We discuss only the homogeneous, isotropic cosmologies described by the Robertson–Walker metric[3],

$$ds^2 = -dt^2 + R^2(t)\left\{\frac{dr^2}{1-kr^2} + r^2(d\theta^2 + \sin^2\theta\, d\phi^2)\right\} \tag{1}$$

where the dynamical evolution of the Universe is expressed by the equation

$$\dot{R}^2 = R^{-1}\left\{\frac{\Lambda R^3}{3} - kR + \frac{8\pi}{3}\rho_0 R_0^3\right\} \tag{2}$$

All symbols have their usual meanings. It is easy to evaluate $\Delta z/(z\Delta\theta)$ by tracing the null geodesics of this geometry. We do this and express the answer in terms of q_0 and Ω_0 by using[4],

$$\begin{aligned}\rho_0 &= \left(\frac{3}{8\pi}\right) H_0^2\,\Omega_0\\ \frac{k}{R_0^2} &= H_0^2\left(\frac{3}{2}\Omega_0 - q_0 - 1\right)\\ \Lambda &= 3H_0^2\left(\frac{1}{2}\Omega_0 - q_0\right)\end{aligned} \tag{3}$$

from which we obtain

$$\begin{aligned}\frac{\Delta z}{z\Delta\theta} = &\frac{\{\frac{1}{2}\Omega_0 - q_0 - (\frac{3}{2}\Omega_0 - q_0 - 1)(1+z)^2 + \Omega_0(1+z)^3\}^{1/2}}{z|\frac{3}{2}\Omega_0 - q_0 - 1|^{1/2}}\\ &\times \sum_k \left(|\tfrac{3}{2}\Omega_0 - q_0 - 1|^{1/2}\int_1^{1+z}\right.\\ &\left.\times\frac{dy}{\{\frac{1}{2}\Omega_0 - q_0 - (\frac{3}{2}\Omega_0 - q_0 - 1)y^2 + \Omega_0 y^3\}^{1/2}}\right)\end{aligned} \tag{4}$$

where

$$\sum_{+1}(x) = \sin x,\ \sum_{-1}(x) = \sinh x \tag{5}$$

In the case $k = 0$,

$$\frac{\Delta z}{z\Delta\theta} = z^{-1}\{1 - \Omega_0 + \Omega_0(1+z)^3\}^{1/2}\int_1^{1+z} dy\,\{1 - \Omega_0 + \Omega_0 y^3\}^{-1/2} \tag{6}$$

For the 'conventional' cosmologies where $\Lambda = 0$ there is the simple expression,

$$\frac{\Delta z}{z\Delta\theta} = \frac{(1+2q_0 z)^{1/2}}{q_0^2 z}\{q_0 z + (q_0 - 1)((1+2q_0 z)^{1/2} - 1)\} \tag{7}$$

Numerical evaluation of equation (7) shows that $\Delta z/(z\Delta\theta)$ is not a powerful estimator of q_0 in the $\Lambda = 0$ case—there is only 11% variation of $\Delta z/(z\Delta\theta)$ between $q_0 = 0$ and $q_0 = 1$ at $z = 2$. However, the general expressions (4) and (6) show great variations of $\Delta z/(z\Delta\theta)$ with the parameters. This is shown in Fig. 1.

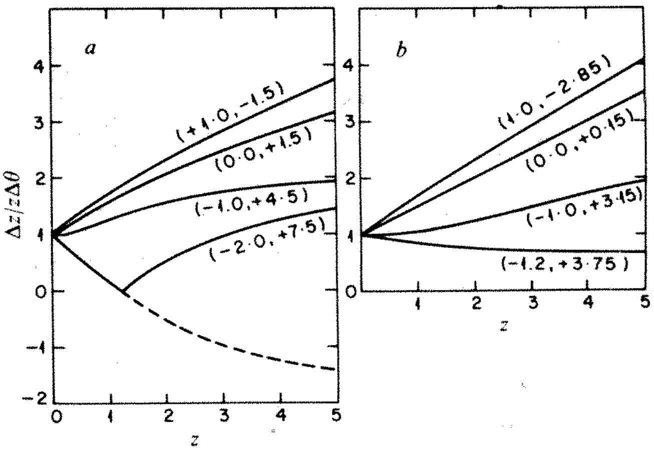

Fig. 1 The variation of $\Delta z/(z\Delta\theta)$ with z is plotted for cosmologies where *a*, $\Omega_0 = 1.0$; *b*, $\Omega_0 = 0.1$. Each curve is labelled by $(q_0, \Lambda H_0^{-2})$ where $\Lambda H_0^{-2} = 1.5\,\Omega_0 - 3\,q_0$. The drastic variations possible in $\Delta z/(z\Delta\theta)$ can be seen even at small z in the event that Λ is significant. The zeros of the lowest curve occur because of antipoles in the null geodesics. The dashed line gives the formal negative result of equation (4), describing apparent reflection of $\Delta\theta$ at the antipoles. The solid curve is the observable result. All of these models have initial singularities. (Note that with $\Omega_0 = 0.1$, $q_0 < -1.28$ means there was no initial singularity.)

The greatest advantage of this method is its independence of the evolutionary corrections which plague most other tests (the proposal to compare X-ray core radii with microwave diminution measurements is a promising exception[5,6]). There is the problem, however, that the natural 'test objects' are galaxies in clusters, which have large peculiar velocities. It will be necessary to separate statistically the peculiar and Hubble velocities in a large number of clusters of different angular extent, but at approximately the same mean redshift. This can be done (in principle) because the Hubble velocities scale $\alpha\ \Delta\theta$, whereas the peculiar velocities will have a very different dependence on $\Delta\theta$.

The analysis will require that there be some clustering which is not gravitationally relaxed. Candidate structures which have been observed at low redshifts might be superclusters[7], or long range correlations around rich clusters[8]. These structures show density enhancements over scales up to $\sim 20\,h^{-1}$ Mpc (where the Hubble constant h is in units of $100\ \mathrm{km\,s^{-1}\,Mpc^{-1}}$). The galaxy two-point correlation function could be measured in z and θ. If the observed correlations have the same shape in z and θ one may be confident that pollution of the Δz by peculiar motions is unimportant. (This is true because, in general, gravitational perturbations produce peculiar velocities with the scaling behaviour $\Delta z \propto \Delta\theta^{1/2}$. This will produce a very different

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*Permanent address: Copernicus Astronomical Center, Polish Academy of Sciences, ul. Bartycka 18, 00–716 Warszawa, Poland.

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shape in the correlation function than the linear relation being searched for.) By matching the amplitudes one constructs a measured $\Delta z/(z\ \Delta\theta)$ which can be used to estimate ΛH_0^{-2}.

It is reasonable to suppose that substantial clustering may occur without the accompanying peculiar velocities. Origins for clustered galaxies have been proposed where, because of dissipation, the peculiar velocities have been damped out. An example is the 'pancake' model[9] where galaxies form on caustics of the gas motion at redshift ≥10.

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Helium gas-bubble superlattice in copper and nickel

P. B. Johnson* & D. J. Mazey

Metallurgy Division, UKAEA AERE Harwell, Oxon, UK

The microstructural changes occurring in metals during ion bombardment with helium or hydrogen isotopes have important implications in several areas of technology[1] such as in the deterioration of the accelerator targets suggested for use as intense neutron sources in cancer therapy[2]; and in the plasma-wall interactions which could strongly influence the operation of future thermonuclear fusion reactors (see ref. 3). Research has shown that helium ion irradiation of Mo[4,5], Cu, Ni and stainless steel[1] to high doses can result in a superlattice of small (~2 nm diameter) helium bubbles in very high concentration (~10^{25} m^{-3}) even at temperatures up to 0.35 T_m (refs 1, 4). The mechanisms involved in the bubble growth[6] and in the superlattice formation have been discussed but the role of displacement damage collisions on bubble growth has received little study. In the present work, specimens containing a superlattice of helium bubbles formed during prior helium ion irradiation have been irradiated with 1 MeV electrons to create a specific collision regime. This was found to induce bubble growth and changes in superlattice parameters, in both copper and nickel.

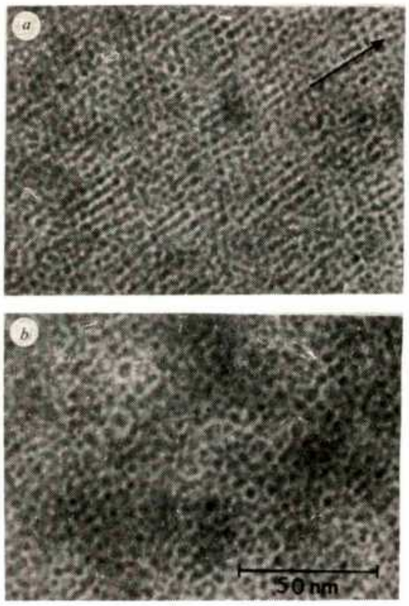

Fig. 1 *a* Direct transmission electron micrograph of helium gas bubbles in copper following helium-ion irradiation to a level of ~4×10^{17} 30 keV He ions cm^{-2} at 300 K. The 100 keV electron beam is along [110] in the matrix and is normal to the plane of the bubble-containing layer. The direction of the trace of one set of (111) matrix planes in the (110) plane is indicated. *b*, Direct transmission electron micrograph of the gas bubbles in the same specimen as *a* but in an area, of diameter ~2μm, which has been irradiated at 300 K with 1 MeV electrons to increase the damage dose by ~15 dpa without the deposition of further helium.

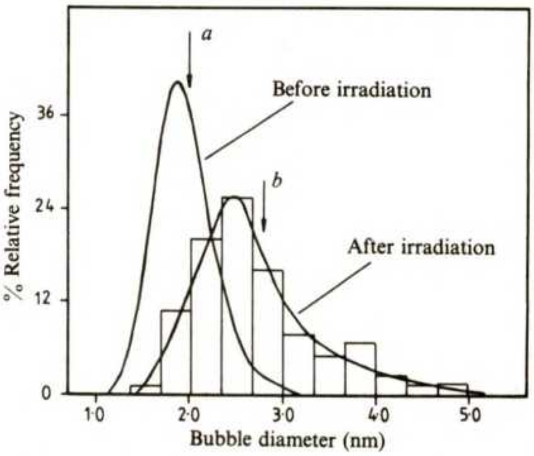

Fig. 2 Bubble size distributions corresponding to Fig. 1*a* and *b*. The average bubble diameters (based on average bubble volumes) indicated by the arrows are ~2.0 nm and ~2.8 nm respectively. It is evident that the 1 MeV electron irradiation has caused a coarsening of the bubble structure.

An annealed copper target was irradiated in bulk at 300 K with 30-keV helium ions at normal incidence and an electron-transparent specimen containing the bubble layer was prepared. The bubbles lay predominantly on a superlattice having an f.c.c. structure aligned with the copper matrix (see Figs 1*a*, 3*a* and ref. 1). The calculated value of the mean projected range was 97 nm with the maximum damage estimated to be ~23 dpa (displacements per atom) at depth ~80 nm. The subsequent 1 MeV electron irradiation at 300 K was carried out on the Harwell AEI HVEM. The damage dose over an irradiated area ~2 μm in diameter was calculated to be 15 dpa and was accumulated in 1 h at a rate similar to that occurring during the ion irradiation at the depth of maximum damage. High resolution microscopy was performed in a Philips EM301 100 keV electron microscope. Electron micrographs taken on, and outside, the 1 MeV electron irradiated area are shown in Fig. 1. For copper, bubble size distributions are shown in Fig. 2, and selected-area diffraction patterns in Fig. 3. It can be seen that following electron irradiation, there is an increase in the average bubble size. A similar result was obtained for nickel in the same conditions. At 300 K the thermal mobility of single vacancies is low in copper and negligible in nickel. The change in the electron diffraction pattern confirms that there has been a genuine change in the bubble structure.

The increase in the bubble lattice constant in copper (from ~7.0 nm to ~9.0 nm) suggests that bubbles may have moved apart under electron irradiation. Between the domains of good order evident in the bubble structure there are regions which seem less well developed[1]. It is possible that local lattice expansions are accommodated by a movement of bubbles outward from the domains into these regions.

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* Permanent address: Department of Physics, Victoria University of Wellington, Wellington, New Zealand.

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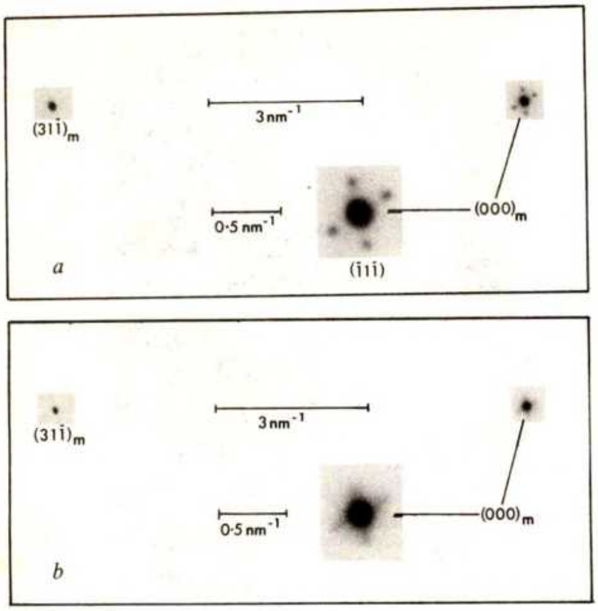

Fig. 3 A section of the [110] selected-area electron diffraction pattern from the copper specimen of Fig. 1. *a* and *b* are respectively outside and on the 1 MeV electron irradiated area. The electron energy is 100 keV. Diffraction from the bubble array causes the splitting that can be seen around the matrix reflections. The superlattice diffraction pattern centred on the (000) transmitted beam is shown enlarged as an inset in each case. Spots which were evident around the (000) reflection in the original negative of *b* have not survived reproduction here. Values for the average spacing between (111) bubble planes, as deduced from the separations of the (111) superlattice spots in the original negatives are ~4.0 nm for *a*, and ~5.2 nm for *b*. (Quantitative results were not obtained for nickel because the patterns were affected by the magnetic nature of the specimen.) The radial streaking evident in *b* shows that in this case there are interplanar spacings ranging from the quoted value through to higher values.

For irradiation at low temperature Evans[6] has suggested that the most likely growth mechanism is one in which overpressurised bubbles punch out interstitial dislocation loops. Assuming that this is the mechanism operating and that no significant long-range movement of the helium takes place during implantation, an estimate can be made of the helium content of the visible bubbles before the electron irradiation (P.B.J. and B. Newport, unpublished). For depths of ~100 nm only ~15% of the helium implanted seems to be in the bubbles. Although the uncertainties involved are large the general conclusion is that an appreciable fraction of the helium is trapped in the matrix probably in the form of helium-vacancy complexes[7]. The estimated density for helium in bubbles (~10^{23} atoms cm^{-3}) exceeds, by a factor ~3, the average value calculated for helium in the matrix at the same depth. This suggests that some helium atoms have moved to the bubbles from their initial place of implantation in the matrix during the course of the helium irradiation. There is no obvious increase in the bubble size once irradiation is stopped. The continued growth during electron irradiation may be in response to bubbles gaining further helium, and perhaps vacancies, displaced from the matrix as a result of electron–atom collisions. It should be emphasised, however, that there are other possibilities which cannot be ruled out.

That collision or displacement events alone can give rise to bubble growth is an important observation in relation to developing an adequate understanding not only of bubble growth during inert gas or proton irradiation but also of synergistic effects when different types of radiation are present, for example in a fusion reactor where the first-wall surface is expected to be bombarded by a variety of ions and particles of widely varying energy[3]. The results presented here could be explained by several different mechanisms acting either individually or in concert. Amongst these are: (1) processes involving the detrapping of helium from helium-vacancy complexes; (2) the radiation assisted migration of helium; (3) the dynamic re-solution of helium from existing bubbles and its redistribution; and (4) the athermal radiation stimulated migration of vacancies to the bubbles[8]. These and other mechanisms will need to be investigated in further experiments to elucidate their roles more precisely.

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Identification of clear taenite in meteorites as ordered FeNi

Edward R. D. Scott

Department of Terrestrial Magnetism, Carnegie Institution of Washington, 5241 Broad Branch Road, N. W., Washington, DC 20015

Roy S. Clarke, Jr

Department of Mineral Sciences, National Museum of Natural History, Smithsonian Institution, Washington, DC 20560

In the Fe–Ni system, the face-centred cubic (f.c.c.) phase (called taenite in meteorites) is stable for all compositions above 1,185 K. Below this temperature, the body-centred cubic phase (kamacite) is stable and has a maximum Ni concentration of about 7% around 720 K (ref. 1). Metal in meteorites is commonly found to contain kamacite with 4–7% Ni and taenite with 20–45% Ni. Taenite grains have M-shaped Ni profiles which formed on slow cooling during diffusion-controlled growth of kamacite and preserve a record of the cooling rates. Another Fe–Ni phase with 49–57% Ni was found in ordinary chondrites by Taylor and Heymann[2] and identified as a variety of taenite. They called it clear taenite to distinguish it from the zoned variety, which develops cloudy borders on etching with acid, and proposed that it nucleated at 660–610 K on the edge of isolated kamacite grains. We report here studies of meteoritic metal with reflected-light microscopy and electron-probe analysis, in which we discovered that clear taenite is optically anisotropic and we have found it in all meteorite groups. We use the term clear taenite as originally defined to include only alloys with ~48–57% Ni, not those with ~25–30% Ni, which Lin *et al.*[3] called clear taenite II. Except for cloudy taenite which contains ~30–40% Ni and looks cloudy because it is a submicroscopic two-phase mixture, taenite remains clear on etching. We conclude that clear taenite is the ordered, tetragonal FeNi phase found by Albertsen *et al.*[4–6] in two iron meteorites using Mössbauer and X-ray techniques, and that it formed from taenite, not kamacite. This tetragonal phase has been synthesised by irradiating FeNi alloys with neutrons or electrons below 593 K (refs 7, 8).

Optical anisotropy in clear taenite is best observed in well polished sections using oil-immersion objectives and a powerful

Table 1 Range and mean composition in wt % of 25 clear taenite grains in eight chondrites and mesosiderites determined by electron-probe analysis

	Range	Mean
Ni	47–57	51
Fe	44–53	49
Cu	0.11–0.38	0.24
Co	0.01–0.8	0.03
P	<0.01	<0.01

source of polarised light. Contrast can often be improved by slightly uncrossing the polars. Clear taenite shows a characteristic pattern of irregular grains (Fig. 1) in which subdomains are usually visible. These subdomains show flame-like or more intricate arrangements and are probably magnetic domains judging from their similarity to those seen in cohenite (Fe_3C) in the same conditions. From the descriptions of Gooley *et al.*[9] it is evident that the mineral in which they found optical anisotropy was in fact Taylor and Heymann's clear taenite. Careful observations of a few large areas of clear taenite reveal three distinct orientations of grains, consistent with the symmetry change from cubic to tetragonal which occurs on ordering in FeNi. Because etching may cause apparent anisotropy, we used only unetched sections to characterise this mineral. In such sections, clear taenite is virtually identical in colour to the zoned taenite which is adjacent, although their interface may sometimes be observed without crossed polars.

In addition to the 10–60-μm diameter grains which Taylor and Heymann[2] identified, clear taenite occurs in sharply defined rims on normal zoned grains. These rims, which are 1–5 μm wide, contain 48–57% Ni and under crossed polars show an irregular grain structure which is characteristic of clear taenite. On etching it can be seen that the anisotropic taenite rim corresponds precisely to the zone of clear taenite between kamacite and cloudy taenite (Fig. 1). Electron probe traverses zoned taenite grains with broad rims in over 10 mesosiderites and chondrites (Figs 2 and 3) show a relatively homogeneous rim and a discontinuity at the interface between clear and cloudy taenite like that found in Estherville[10]. These features in the M-profile were not resolved by other workers (for example, refs 11, 12) because they did not analyse small favourably located grains with broad rims.

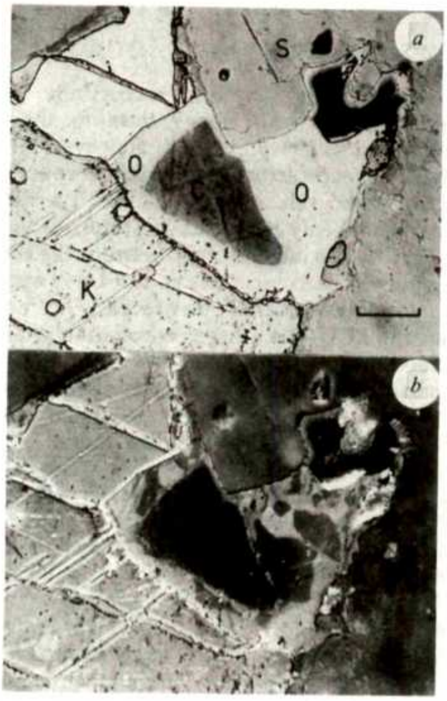

Fig. 1 Reflected light photomicrograph of metal grain in Mezö-Madaras, an L3 meteorite, etched with acid to reveal structure; *a*, polarised light; *b*, crossed polars. In *a* cloudy taenite (C) is surrounded by a white rim of clear taenite (O). Crossed polars show that clear taenite is optically anisotropic and reveal an irregular grain structure. Clear taenite is surrounded by kamacite (K) and silicate (S). (Polished section is carbon coated; this enhances contrast between grains in clear taenite by reducing the visibility of the subdomains.) Scale bar, 20 μm.

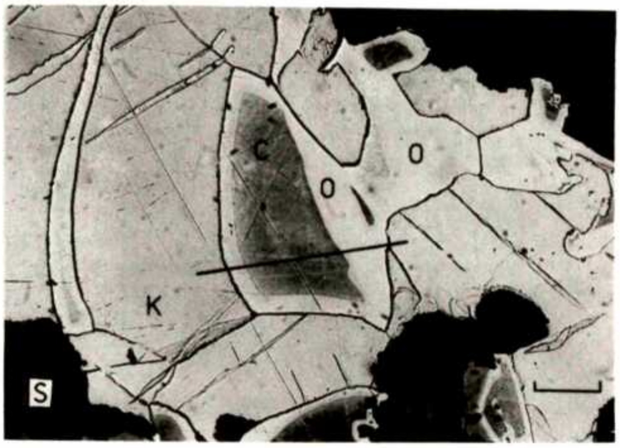

Fig. 2 Reflected light micrograph of metal grain in Mezö-Madaras. The line across the cloudy taenite grain (C) which is rimmed by ordered clear taenite (O) marks the location of the compositional profile shown in Fig. 3. Kamacite, K; silicate, S; scale bar, 20 μm.

We have found optical anisotropy in clear taenite from over 40 meteorites including H, L, LL and CO3 chondrites, diogenites, iron meteorites, mesosiderites and pallasites and conclude that all slowly cooled meteorites with zoned taenite contain some clear, anisotropic taenite also. Table 1 summarises the compositions of clear taenite found by electron-probe analysis of eight meteorites. With the exception of reheated meteorites and heat-affected surfaces we have not found normal isotropic taenite with compositions in the range 46–60% Ni.

The intimate association of clear taenite with zoned taenite and its growth on grain boundaries within polycrystalline taenite show that few clear taenite grains could have formed in isolated kamacite grains as Taylor and Heymann[2] suggested. Instead we propose that large clear taenite, which is found in ordinary chondrites and mesosiderites, formed by the inward growth of clear taenite rims at low temperatures (<700 K). In the mesosiderites Estherville and Emery and the L chondrite Mezö-Madaras, we have seen complete sequences of structures ranging from large clear taenite grains enclosing micrometre-sized blobs of cloudy taenite to large-zoned grains with micrometre-wide rims of clear taenite enclosing cloudy taenite. In most chondrites the intermediate stages in this sequence are less common and there seem to be two distinct populations of grains. Diffusion of Ni through up to 30 μm of clear taenite, which is required in our mechanism, may be facilitated by the numerous grain boundaries.

Even in pallasites and slowly cooled iron meteorites, massive clear taenite grains are absent and the clear rims on zoned grains are relatively narrow compared with those in chondrites. This may be a result of relatively high metallic concentrations of P, which reduce the equilibrium concentration of Ni in taenite[13]. Taenite with about 50% Ni has also been reported in shocked sulphides in irons[14]. Other processes must be responsible for its formation there.

The anisotropic FeNi mineral which we have described is undoubtedly the ordered phase reported by Albertsen *et al.*[5,6] in two iron meteorites. They identified it by the similarity of its Mössbauer spectrum to that of tetragonal FeNi synthesised with neutron irradiation[7,8] and the presence of X-ray reflections

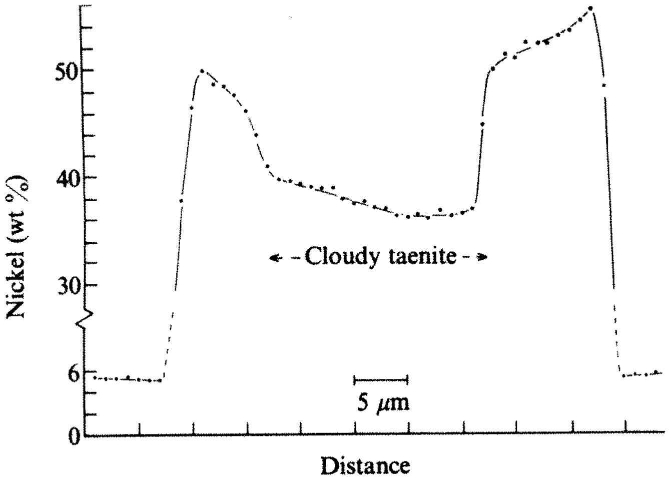

Fig. 3 Electron probe Ni profile across composite taenite grain shown in Fig. 2. The Ni profile is not smooth as others have found; instead there are discontinuities between the clear taenite rim which has 46–55% Ni and the cloudy taenite which has less than 40% Ni.

which violated the f.c.c. lattice of taenite. The only possible ordering arrangement which satisfied their data was the tetragonal L10 structure (AuCu), which can be derived from taenite by ordering Fe and Ni atoms on alternate (002) planes. From etching experiments on zoned taenite lamellae, Albertsen *et al.*[6] deduced that FeNi was concentrated in the outer rims of these lamellae. Our observations and probe analyses of anisotropic rims are entirely consistent with their work, and taken together they characterise clear taenite containing 48–57% Ni as a new mineral.

Ordered FeNi areas several micrometres in size have also been reported[15,16] in another iron meteorite, Santa Catharina, which contains 34% Ni (ref. 14). Nickel could not have been concentrated in this iron by growth of kamacite, which is virtually absent; instead, local oxidation of Fe has been suggested as a mechanism by Bowles *et al.*[15] but refuted by Danon *et al.*[16]. Although the meteorites in which we observed clear taenite are not all entirely unoxidised, the areas which we studied were free from visible oxidation.

As the phases in cloudy taenite can only be resolved by electron microscopy[3,10] we cannot confirm that cloudy taenite also contains ordered FeNi (refs 4, 5). It is plausible that exsolution in the cloudy zone is triggered by ordering of FeNi, possibly through spinodal decomposition. Electron microscopy of Santa Catharina by Jago[17] did show two submicrometre taenite phases in a structure which seemed to be a precursor to cloudy taenite. However he detected extra reflections consistent with ordering in taenite (and kamacite) only after the second taenite had disappeared on heating at 200 °C.

Estimates of cooling rates[11,12] ignore the existence of ordered FeNi rims and the two-phase nature of cloudy taenite. Although this is unlikely to cause large errors, a quantitative investigation of these effects is needed. The abundances of clear and cloudy taenites provide a useful visual estimate of the cooling rate as there are rough inverse correlations between abundances and cooling rates.

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On the total CO_2–titration alkalinity–oxygen system in the Pacific Ocean

Chen-Tung A. Chen & Ricardo M. Pytkowicz

School of Oceanography, Oregon State University, Corvallis, Oregon 97331

Decomposition of organic matter changes the concentrations of carbon, nitrogen, phosphorus, oxygen and titration alkalinity (TA) in the ratio 106:16:1:138:–17 (refs 1–3), so the combined effect of decomposing *x* mol of $CaCO_3$ and *y* mol of organic matter in 1 kg of seawater on the preformed total CO_2 (ΣCO_2^0), preformed TA (TA^0), biogenerated ΣCO_2 [$\Delta\Sigma CO_2$(biol)] and the apparent oxygen utilisation (AOU) can be represented as[4]

$$\Delta\Sigma CO_2 = \Sigma CO_2(\text{measured}) - \Sigma CO_2^0 = x + 106y$$

$$\Delta TA = TA(\text{measured}) - TA^0 = 2x - 17y$$

$$\Delta\Sigma CO_2(\text{biol}) = 106y; \qquad AOU = 138y$$

Eliminating *x* and *y* from the above equations yields

$$\Delta\Sigma CO_2(\text{biol}) = 0.768\ AOU \tag{1}$$

$$\Delta\Sigma CO_2 - 0.5\Delta TA = 0.83\ AOU \tag{2}$$

Several early workers[5–8] have successfully correlated $\Delta\Sigma CO_2$(biol) to AOU with slopes close to the Redfield, Ketchum and Richards (RKR) model[1]. However, the methods used often have not satisfactorily taken into account the variation of the preformed values for ΣCO_2 and TA with sample depth or have mistakenly taken $\Delta\Sigma CO_2$(biol) to be equal to $\Delta\Sigma CO_2 - 0.5\Delta TA$. The inconsistency between the correlations above and below the thermocline is also not well explained. A modified computational scheme[5,8] has recently been developed for calculating the ΣCO_2-TA-oxygen correlation with the depth dependent variations of ΣCO_2^0 and TA^0 accounted for. The method of calculating $\Delta\Sigma CO_2$ is the same as that described in ref. 4 but further useful information has been obtained by plotting $\Delta\Sigma CO_2$ against AOU, rather than depth. The results in the Pacific Ocean, presented here, suggest a linear correlation between the bio-generated CO_2 and AOU with a slope of 0.722 ± 0.05, in good agreement with that predicted from the RKR model. The deviation of the data from this linear correlation for shallow water can largely be explained by the influences of human induced CO_2.

The values for ΣCO_2^0 and TA^0 are needed in order to calculate $\Delta\Sigma CO_2$ and ΔTA. It has been shown[8,9] that the surface values of ΣCO_2 and TA show a linear correlation with temperature. Using more recent data (Geochemical Ocean Section Study, Atlantic Ocean), Chen and Millero[4] have confirmed that TA^0 and ΣCO_2^0 show a linear temperature dependence on the constant salinity basis, but only at the lower temperatures. We have, therefore, normalised the values for ΣCO_2^0 and TA^0 in the Pacific Ocean (GEOSECS) to 35‰ salinity using

$$\Sigma CO_2^0(\text{or } TA^0) = \frac{\Sigma CO_2^0(\text{measured}) \ [\text{or } TA^0(\text{measured})] \times 35.000}{S(‰, \text{measured})}$$

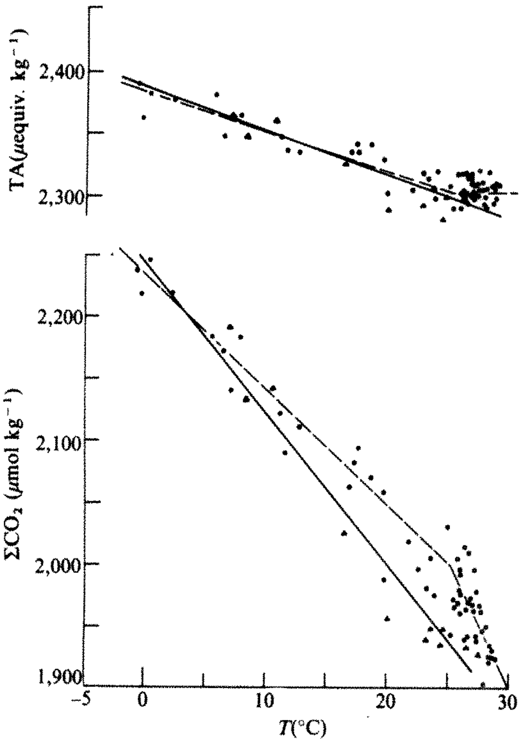

Fig. 1 The surface TA and ΣCO_2 values, normalised to 35‰ salinity, at various temperatures. The triangles and the solid line represent the data in the Northwest Pacific. The circles and the dashed line represent the data elsewhere in the Pacific Ocean.

These normalised values also correlate with the potential temperature in the Pacific Ocean although they seem to fall in two groups (Fig. 1). Stations in the north-west region (shaded area on Fig. 2) seem to have different values for ΣCO_2^0 and TA^0 than the rest of the Pacific Ocean. As a result, the following equations are used to represent ΣCO_2^0 and TA^0 in the north-west region (triangles, represented by solid lines on Fig. 1):

$$\begin{aligned} \Sigma CO_2^0(\mu\text{mol kg}^{-1}) &= 2{,}242 - 12.08\theta\ (\pm 18) \\ TA^0(\mu\text{equiv kg}^{-1}) &= 2{,}384 - 3.36\theta\ (\pm 11) \end{aligned} \qquad (3)$$

Whereas, the following equations are used to represent the values elsewhere in the Pacific (circles, represented by dashed lines on Fig. 1):

$$\begin{aligned} \Sigma CO_2^0(\mu\text{mol kg}^{-1}) &= 2{,}236 - 9.4\theta\ (\pm 21) \qquad \theta \leq 25\,°\text{C} \\ &= 2{,}500 - 20\theta\ (\pm 23) \qquad \theta > 25\,°\text{C} \end{aligned} \qquad (4)$$

$$\begin{aligned} TA^0(\mu\text{equiv kg}^{-1}) &= 2{,}383 - 3.1\theta\ (\pm 10) \qquad \theta \leq 25\,°\text{C} \\ &= 2{,}306 \qquad\quad (\pm 10) \qquad \theta > 25\,°\text{C} \end{aligned} \qquad (5)$$

The numbers in parentheses give one standard deviation of the least squares fit for the above equations. The salinity and higher order temperatures terms are not statistically significant.

The GEOSECS station GS 347 has been chosen as a demonstration station. The values for $\Delta\Sigma CO_2 - 0.5\Delta TA$ calculated from equations (2)–(5) are plotted against AOU in Fig. 3. The dashed line (*a*) on Fig. 3 has a slope of 0.83, therefore, one might expect the data points to fall on the line if both the computational scheme and the RKR model are correct. This is clearly not the case. Although the sample at 20 m falls near the theoretical line, that at 189 m falls away from it and that at 336 m falls even further away. The samples below 589 m deviate from line *a* the most but seem to correlate linearly with AOU (line *b*) with a slope of 0.76 ± 0.05 similar to that predicted by the RKR model.

Two possibilities may explain the phenomenon, which is typical for the stations we examined. First, that the RKR model is invalid in the near-surface layer but becomes valid for deep waters—this seems unreasonable. Second, there may be a deficiency in our computational scheme—the values of present-day ΣCO_2^0 used to calculate $\Delta\Sigma CO_2$ might not represent the values of ΣCO_2^0 for waters formed in the past.

Human activities, mainly the burning of fossil fuels and possibly also the cultivation of virgin lands and clearing of forests, have significantly increased the amount of CO_2 in the atmosphere over the last 130 years[10–18]. Recent work[4,19–21] has shown that the human-induced CO_2 has penetrated into the oceans. Consequently, the ΣCO_2^0 values calculated from the present-day data probably are higher than the ΣCO_2^0 values for old waters. The values for TA^0 probably do not vary with atmospheric CO_2 and we will assume that the present-day TA^0 values represent the values of TA^0 for water of various ages in our discussion.

Since the values of ΣCO_2^0 for younger waters are closer to the present-day ΣCO_2^0 values (equations (3) and (4)), the computational scheme is 'more correct' for younger waters. This agrees with the data shown on Fig. 3; the shallower, and presumably, younger samples fall closer to the theoretical line. The waters below 589 m deviate from the line by a nearly constant amount which indicates that the ΣCO_2^0 values for these waters deviate from the present day ΣCO_2^0 by approximately a constant amount. In other words, the waters below 589 m probably have not felt the influence of the atmospheric CO_2 variations. Although the ^{14}C and tritium have different source function and equilibration time as compared to CO_2, their vertical distributions[22] seem to substantiate this conclusion, at least qualitatively (Fig. 3). Very little tritium, but large negative values for $\Delta^{14}C$ have been found below 590 m.

It is generally accepted that protein is utilised more readily than carbohydrate as particulate organic matter is transported from surface to deep waters[23–26], consequently, the C/N/P/O ratio changes accordingly. However, our results indicate that since the changes in the masses of nitrogen and phosphorus are small as compared to the changes in the masses of carbon and oxygen, the changes in the C/N, C/P, N/P, N/O and P/O ratios probably do not significantly affect the C/O ratio. The preferential retention of carbon over oxygen in upper waters could indeed change the C/O ratio. However, this process has to be significantly effective everywhere from surface to 589 m deep. No known process and no experimental data support this. As a result, the increase of ΣCO_2 due to the bioregeneration correlates linearly with AOU (line *b* on Fig. 3) below 589 m with a slope close to the value predicted by the RKR model.

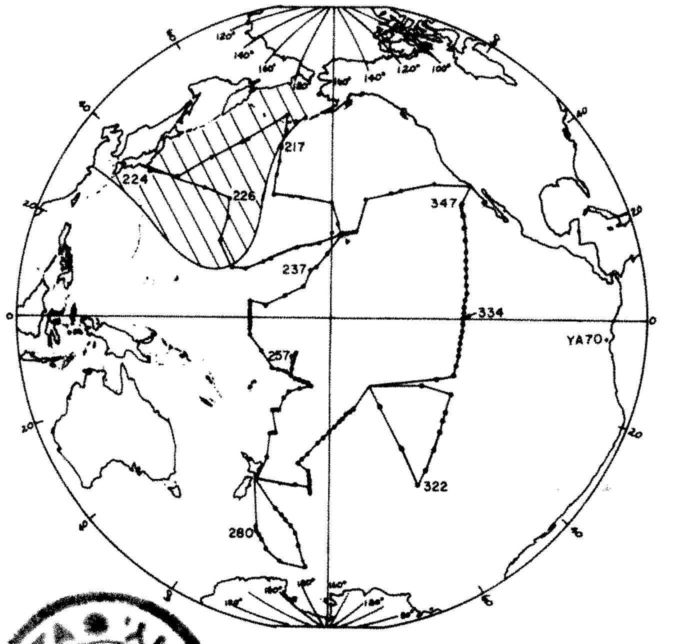

Fig. 2 Station locations in the Pacific Ocean.

The deviation of the data from the predicted values in the upper layer probably is largely caused by the influx of human induced CO_2. The best linear fit of the values for $\Delta\Sigma CO_2 - 0.5\Delta TA$ gives a value of $41 \pm 15\ \mu mol\ kg^{-1}$ (the distance between O and A) at AOU = 0. The value of $41 \pm 15\ \mu mol\ kg^{-1}$ probably represents the change in ΣCO_2^0 ($\Delta\Sigma CO_2^0$) due to the change of CO_2 concentration in the atmosphere. (The uncertainty of $\pm 15\ \mu mol\ kg^{-1}$ reflects only the random error due to scatter of the observed data and does not include any systematic error.) Integrating $\Delta\Sigma CO_2^0$ from surface to 589 m gives a total CO_2 increase of $1.2\ m\,mol\ cm^{-2}$. As a very crude first approximation, we assumed that the average difference in the concentration of man-made ΣCO_2 was $5 \pm 5\ \mu mol\ kg^{-1}$ between surface and 589 m over the last 130 yr and arrived at an eddy diffusion coefficient of $3.5 \pm 3.5\ cm^2\ s^{-1}$. The value is in reasonable agreement with the findings of Pytkowicz for oxygen[27] and Oeschger *et al.* for CO_2[28].

Data from stations widely scattered in the Pacific Ocean (Fig. 2; GS 217, 224, 226, 237, 257, 280, 322, 334, 347 and YALOC 69 station 70) have also been examined and the results follow the same pattern as GS 347. The values for $\Delta\Sigma CO_2 - 0.5\Delta TA$ for these stations have been plotted against AOU in Fig. 4. These values from the deep layers (filled circles; depth varies from below 100 m at YA 70 to below 1200 m at GS 226 and 322) correlate linearly (line *b*) with AOU with an average slope of 0.78 ± 0.05, which corresponds to a slope of 0.722 between $\Delta\Sigma CO_2$ (biol) and AOU. The data from the shallower layers (open circles) deviate from this best fit straight line with the near surface values centred around (0, 0) interception. Line *a* has the same slope as line *b* but is drawn through the (0, 0) interception. The vertical distance between a data point and line *a* probably represents the difference between the previous and present ocean surface ΣCO_2 concentrations.

Note that our computational scheme represents only first approximations of the very complicated system in the oceans[4]. Although the decrease of TA^0 and ΣCO_2^0 with increasing temperatures can be qualitatively explained as due to the decrease in the solubility for $CaCO_3$ and CO_2, the magnitude of the decrease cannot yet be estimated theoretically. There is also no satisfactory explanation as to why the correlations of TA^0 and ΣCO_2^0 with temperature change slope at higher temperatures. Whether they are caused by the change in the biological activity, or by the change in current flow patterns, or other physical processes such as equatorial upwelling[29] deserves further investigation. Moreover, the GEOSECS summer data in the Pacific Ocean have been used in our calculations although the winter TA^0 and ΣCO_2^0 concentrations in the Atlantic and Pacific polar regions where intensive vertical mixing occurs may be different from the Pacific summer data. This summer–winter incompatibility may cause a systematic error in addition to the random error. However, our preliminary estimation indicates that the $\Delta\Sigma CO_2^0$ value calculated here is unlikely to be systematically too high by more than $15\ \mu mol\ kg^{-1}$. A maximum value of $26\ \mu mol\ kg^{-1}$ in $\Delta\Sigma CO_2^0$ would give an eddy diffusion coefficient of $2.2 \pm 2.2\ cm^2\ s^{-1}$, which is in good agreement with the results of Pytkowicz[27] and Oeschger *et al.*[28]. The slope of the $\Delta\Sigma CO_2 - 0.5\ \Delta TA$ against AOU correlation would remain essentially the same, however.

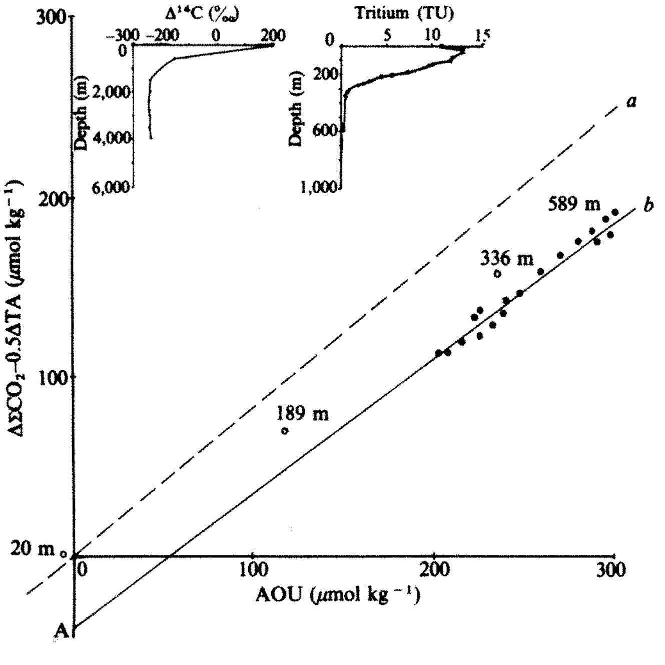

Fig. 3 The relationship between $\Delta\Sigma CO_2 - 0.5\Delta TA$ and AOU at GS 347. Open circles denote young waters and filled circles, old waters. Line *a* represents the theoretical correlation predicted from the RKR model. Line *b* is the best-fit correlation for the old water samples. The insert shows the tritium (in tritium units, TU) and $\Delta^{14}C$ (‰) profiles.

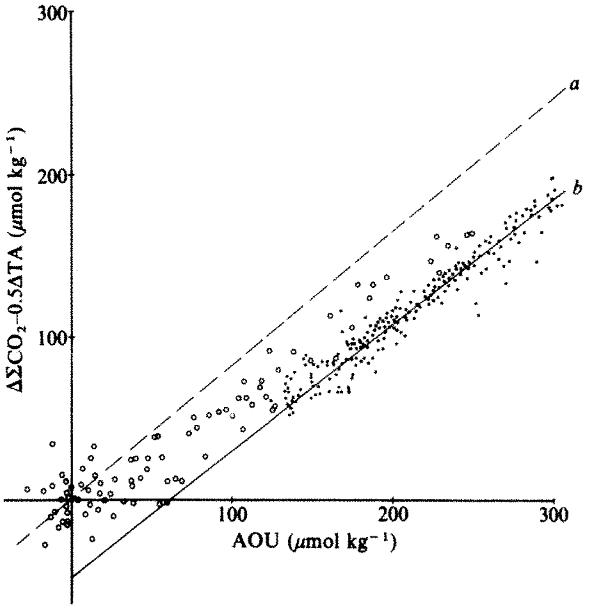

Fig. 4 The relationship between $\Delta\Sigma CO_2 - 0.5\Delta TA$ and AOU at 10 stations in the Pacific Ocean. Line *b* is the best fit straight line for the old water samples (filled circles). Line *a* has the same slope as line *b* but is drawn through the (0, 0) interception.

These data were obtained as part of the Pacific GEOSECS Expedition carried out aboard RV Melville of the Scripps Institution of Oceanography during 1973 and 1974. The shipboard work was largely carried out by personnel of the GEOSECS Operations Group under the direction of the late Arnold E. Bainbridge.

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Primitive lead in deep crustal xenoliths from the Snake River Plain, Idaho

William P. Leeman

Department of Geology, Rice University, Houston, Texas 77001

The petrology and geochemistry of deep crustal rocks are poorly understood due to the inaccessibility of such materials. Most inferences of the lower crust are based on studies of ancient high-grade metamorphic terranes now exposed after a history of deep burial. However, direct sampling of deep crustal rocks is feasible when they are carried to the surface as accidental fragments (or xenoliths) in lava flows. Ferro-basalt to ferro-latite lavas from Craters of the Moon lava field (COM) in the Snake River Plain, southern Idaho are characterised by variable and high $^{87}Sr/^{86}Sr$ ratios (0.708–0.712) interpreted as reflecting variable degrees of crustal contamination[1,2]. Some of these lavas contain xenoliths of crustal derivation (silicic charnockites and pyroxene granulites) that are found nowhere in the vicinity in surface outcrops. I present here initial isotopic and trace element results for a suite of these crustal xenoliths. As part of a detailed Pb isotopic study of the lavas (in preparation), three granulites were analysed to test the contamination hypothesis. The results indicate an affinity between the xenoliths and many exposed granulite-facies metamorphic terranes, and the unusually primitive lead isotopic ratios recorded establish the presence of early Archean crust beneath south-central Idaho.

The crustal xenoliths all contain quartz, plagioclase, Fe–Ti oxides, and hypersthene ± clinopyroxene ± alkali feldspar. These mineral assemblages place the rocks in the granulite facies at intermediate crustal depths. Three fresh xenoliths, 15–50 cm in approximate diameter, were sampled by drilling 1.2-cm cores, weighing about 30–50 g, from central portions of the rocks. In one case a core was taken near the xenolith rim to test for contamination from the host lava. The cores were washed in dilute HCl and deionised triple-distilled H_2O, crushed in a WC mill, digested in HF–$HC10_4$ in Teflon beakers in a low-Pb laboratory. Pb was separated by a $Ba(NO_3)_2$ precipitation and purified by anodic electrodeposition. Pb isotopic compositions and trace element concentrations were determined on separate dissolutions. Analyses were made on a 30 cm NBS-design mass spectrometer using the Si-gel method and isotopic compositions were corrected for mass fractionation by the double-spiking method[3]. Blanks run with these samples averaged about 5 ng and are insignificant for the reported analyses. All isotopic ratios are accurate to about 0.15% relative to NBS standard SRM-981. Pb, U, Th, Rb and Sr concentrations were determined by isotope dilution methods and are accurate to within 3% of the amount present. Table 1 shows replicate analyses (full procedural duplicates).

Isotopic compositions of the xenoliths are extremely primitive and are quite distinct from the host and all other analysed COM lavas. Core and rim analyses of xenolith CKI-1 are marginally distinct, but in fact the rim sample is the least radiogenic and seemingly precludes addition of Pb to the xenolith from the host lava. Isotopic ratios for this xenolith are among the least radiogenic values reported from the US. The $^{206}Pb/^{204}Pb$ and $^{207}Pb/^{204}Pb$ ratios plot near the 2,900 Myr isochron through the Stacey–Kramers growth curve for conformable ore leads[4] and

Table 1 Isotopic and trace element data for COM granulite xenoliths*

K	Rb	Sr	Pb	U	Th	Rb/Sr	Th/U	μ	$^{87}Sr/^{86}Sr$	$^{206}Pb/^{204}Pb$	$^{207}Pb/^{204}Pb$	$^{208}Pb/^{204}Pb$
V-31 Devils Orchard flow (ferro-latite)												
3.88	97	175	31.1	3.25	11.8	0.554	3.6	6.59	0.7110	17.81	15.60	38.51
										17.82	15.62	38.56
										17.81	15.60	38.52†
SI-1 charnockite												
0.86	6.34	69.8	9.02	0.36	33.2	0.091	92.2	3.17	0.7173	16.08	15.62	58.97
										16.11	15.70	59.26
										16.09	15.64	59.07
70-40 hypersthene granulite												
2.01	22.2	325	22.4	<0.2	6.09	0.068	>30	<0.50	0.7152	14.12	14.84	35.77
										14.09	14.81	35.70
										14.11	14.82	35.73
CKI-1 charnockite (core)												
2.18	30.0	235	15.8	0.061	0.104	0.128	1.6	0.215	0.7271	13.55	14.70	35.00
										13.56	14.73	35.03
										13.56	14.72	35.01
CKI-1 (rim)												
—	—	—	16.3	—	—	—	—	—	—	13.42	14.67	34.65
										13.44	14.70	34.75
										13.43	14.68	34.70

Internal precision (2σ) on isotopic ratios is <0.0003 (87/86), <0.02 (206/204), <0.03 (207/204), and <0.06 (208/204) for typical analyses.

* All concentrations in p.p.m. except K (%).

† Weighted mean for the replecate analysis, considering analytical errors for the individual ratios.

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indicate that CKI-1 represents a terrane at least that old. Other reasonable growth curves (for example, a single-stage growth curve with a ^{238}U/^{204}Pb value of about 8.5 and Th/U value of about 6) yield model lead isotopic ratios similar to those in CKI-1 at about 2,900 Myr ago. The actual age of the xenolith (or strictly speaking, the age of granulite metamorphism) is probably slightly greater than 2,900 Myr because no correction has been applied to the observed isotopic ratios to account for *in situ* decay of U and Th isotopes. This correction is not large for CKI-1 due to its low U and Th contents. Acid leaching studies are in progress to estimate more closely the initial lead isotopic composition of this rock. The other xenoliths are somewhat more radiogenic than CKI-1 in accordance with their higher U and Th contents. The model age for CKI-1 is supported by a ^{207}Pb/^{204}Pb–^{206}Pb/^{204}Pb secondary isochron age of 2,870 Myr for the pair of xenoliths, CKI-1 and 70–40. However, a secondary isochron age of 3,760 Myr is indicated for the pair, SI-1 and CKI-1; this result probably reflects complex pre-metamorphic evolution of the lead in these two rocks. Further work is required to define the pre-metamorphic history of the COM xenoliths.

All of the xenoliths have high K/Rb ratios (730 to 1,360) and very low U contents and two have low Th contents. These geochemical features are characteristic of high-grade metamorphic terranes[5]. Considering their primitive lead isotopic compositions, it is clear that the low U and Th contents reflect depletions of these elements during an ancient metamorphic event and cannot have resulted from interaction with the host lavas. Thus it is clear that these few samples record the presence of a deep Archean basement complex at least several hundred kilometres west of other known outcrops in the northern Rocky Mountains. Archean metasedimentary rocks (2,500 Myr) occur south of the Snake River Plain in the Albion Range[6], but these are of a lower metamorphic grade than the COM xenoliths.

Sr isotropic ratios for the COM xenoliths are high and, with the low Rb/Sr ratios, provide a further indication of the antiquity of the underlying crust. The few available Rb-Sr data[1] are too scattered to provide a whole-rock isochron. If the model lead age is accepted, it is likely that the xenoliths were characterised by varied initial ^{87}Sr/^{86}Sr ratios unless the Rb-Sr systematics have been disturbed.

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Biopedological origin of peatlands in South East Alaska

F. C. Ugolini & D. H. Mann

College of Forest Resources, University of Washington, Seattle, Washington 98195

Peatlands are the final stage in plant succession on level surfaces in South East Alaska, a region where bogs and forests in the process of turning into bogs occupy nearly as much surface area as do forests proper[1–3]. Plant succession in this area is in part conditioned by progressive bogging[4]; however, no previous attempts have been made to relate paludification to soil development. We now propose that the formation of peatlands in the Lituya Bay area of South East Alaska is caused by the deterioration of the internal drainage of the soil as an iron-cemented pan develops during the process of podzolisation. This situation provides a clear example of soil-induced bog formation, a process that may be important in other parts of the world.

Along the coast of the Gulf of Alaska, in Glacier Bay National Monument, tectonic uplift along the active Fairweather Fault and eustatic sea-level changes have produced marine terraces (Fig. 1). The wave-planed, seaward-sloping bedrock of the terraces is covered by 2–4 m of beach sands and gravels. The terraces provide a chronosequence of surfaces on which plant communities and soils have developed.

Terrace A, the lowest, is 150 m wide with an average elevation of 9 m above sea level; its minimum age is estimated at 400 yr from the ages of the oldest trees (Fig. 2). Terrace B is approximately 250 m wide with an average elevation of 26 m; its estimated age is $2\text{–}3 \times 10^3$ yr (ref. 5). Terrace C is 1,600 m wide with an average elevation of 80 m, and is estimated to be $6\text{-}8 \times 10^3$ yr old[5].

We investigated five transects across these terraces near Lituya Bay. For each transect, soils and the vegetation were described, and the ages of the largest trees determined by coring. We will describe here the results from a representative transect, the Steelhead Transect, 10 km south-east of Lituya Bay (Figs 1, 2).

The seaward edge of terrace A is covered by rye grass (*Elymus arenarius*) and supports immature soils with A/C sequences (regosols or typic udipsamments). A Sitka spruce (*Picea sitchensis*) forest occupies the inland side of this terrace, and the soil has weakly developed B horizons with A(B)C profiles.

The seaward side of terrace B is covered by Sitka spruce with western hemlock (*Tsuga heterophylla*) in the understory. Soils on the seaward side are mature typic haplorthods or placorthods (podzols) with an A2, a friable B2hir and a firm or cemented B2ir horizon. The inland side of terrace B is dominated by western hemlock and Alaska yellow cedar (*Chamaecyparis nootkatensis*). The soils exhibit thick organic horizons, impervious B horizons (placic horizons) and impeded drainage.

Alaska yellow cedar and mountain hemlock (*Tsuga mertensiana*) form a stunted forest on the seaward edge of terrace C. Soils in this forest are histosols (peat soils) with some spodic (podzolic) characteristics, but the organic horizons are in excess of 1 m and extremely poorly drained. The rest of terrace C is occupied by peatland vegetation dominated by sphagnum, sedges and ericaceous shrubs growing on 1.5–2.0 m of well decomposed peat. This peat lies on a thin (15 cm), compacted, iron-stained layer, rich in woody organics, resting on sand which shows no morphological or chemical evidence of B2hir or B2ir horizons. Biotic remains in this profile indicate a successional change from forest to peatland. The basal woody layer contains remains of a carabid beetle, *Patrobus fossifrons*, which normally does not inhabit sphagnum bogs[6]; abruptly above this level seeds of the bog plant *Menyathes trifoliata* appear.

The successional sequence is further described by the following chemical, physical and biological parameters of the soil. With distance from the sea, the organic horizon thickness increases from 2 to 20 cm across terrace A and from 22 to 35 cm across terrace B. On terrace C, a 100-fold increase occurs across the stunted forest/peatland boundary. The abundance of collembola and mites in organic horizons provides an index of the decomposition due to aerobic organisms. These soil arthro-

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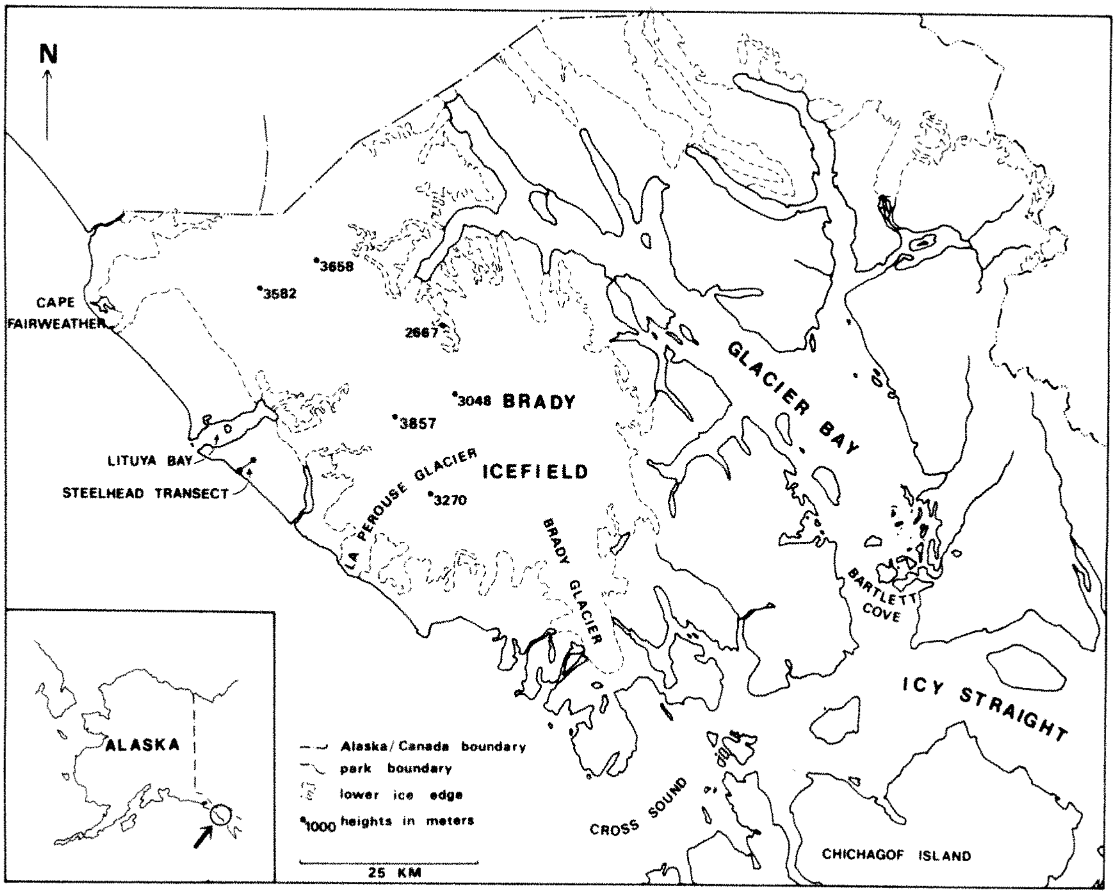

Fig. 1 Map of Glacier Bay National Monument, Alaska, showing the Lituya Bay and the location of study site.

pods disappear rapidly inland along the transect, reaching lowest numbers in the stunted forest of terrace C. The *p*H decreases with increasing age and development of the soil. It decreases 2.5 units between the beach meadow and the bog, the greatest drop occurring at the start of the conifer forest. The amount of iron in the B horizon, as crystalline, inorganic amorphous, and organically complexed, parallels the soil development; it increases from 0.16% in the beach meadow to a maximum of 1.84% in the B2hir of the seaward side of terrace B. It decreases rapidly on terrace C as the soils become more poorly drained. The organically complexed iron increases from 0.60% in the B2 horizon at the rear of terrace A to a maximum of 1.00% in the B2ir of the seaward side of terrace B. Such iron is progressively depleted at the rear of terrace B and on terrace C. Virtually the only form of iron left in the profile at the bog site is organically complexed and associated with the basal layers of woody peat. The log of saturated flow of intact soil cores decreases from 1 ml min^{-1} cm^{-2} in the B2 of terrace A to −3.5 ml min^{-1} cm^{-2} in the B2hir of terrace C. Field observations revealed an abundant flow of water along the surface of the B2hir in several profiles on terraces B and C.

The above observations clearly show that the terraces near Lituya Bay comprise a soil and vegetation chronosequence spanning about 8,000 yr and telescoped into an horizontal distance of less than 1 km. The rapid rates of both soil formation and plant succession are favoured by the maritime climate of South East Alaska which allows an unusually long growing season for this latitude[7]. Heavy precipitation (280 cm) and low evapo-transpiration encourage lush plant growth and rapid mineral weathering.

The terrace chronosequence near Lituya Bay offers a new perspective on the interaction between soil development, paludification and plant succession in South East Alaska. We propose the following sequence of events for peatland development on the terraces south-east of Lituya Bay (Fig. 3). (1) Soil-forming processes initiate the development of genetic horizons on surfaces newly emerged from the sea. (2) The accumulation of sesquioxides in the B horizon, induced by podzolisation, results in the formation of placic horizons. In South East Alaska placic horizons are known to appear after about 500 yr[8]. (3) Placic horizons cause deterioration of the soil's internal drainage, creating anaerobic conditions in the overlying horizons with attendant lowering of the decomposition rate of organic matter in the forest floor. Field observations show that placic horizons form an effective barrier to plant roots. (4) Because of slowed decomposition, litter accumulation on the forest floor proceeds rapidly. (5) At some point, equivalent to the stunted forest zone of terrace C, the litter layer surpasses a critical mass and becomes capable of retaining water the whole year round to maintain anaerobic conditions without the help of impervious B horizons. (6) In the face of continuous anaerobic conditions, the iron-cementing agents of the placic B horizons become reduced and removed in the groundwater. (7) Organic acids have lowered the *p*H and conditions are right for

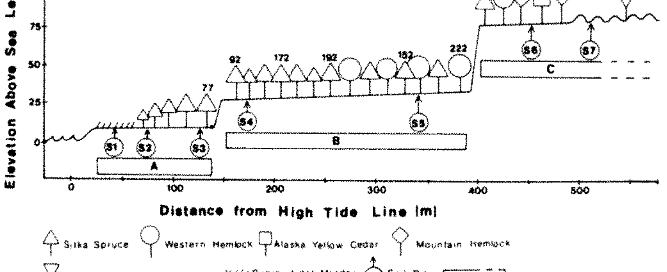

Fig. 2 Cross-section of the Steelhead Transect showing soil pit locations and tree ages across the raised terraces.

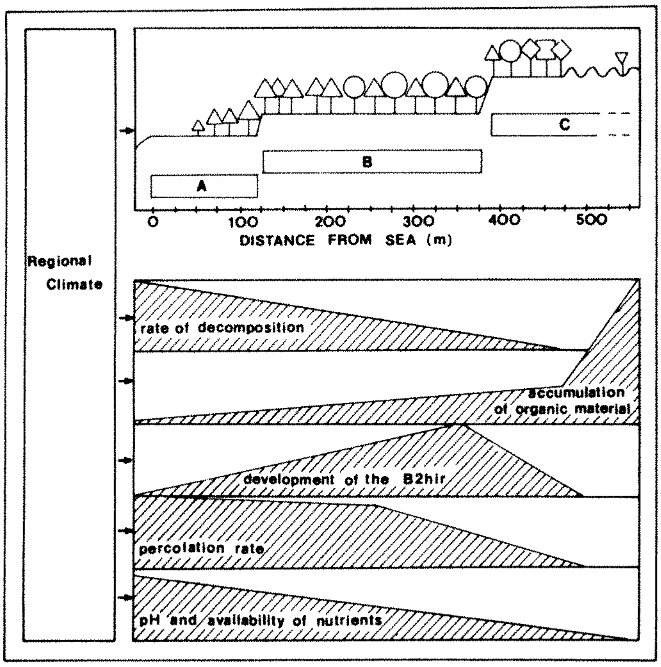

Fig. 3 A model of bog formation near Lituya Bay, South East Alaska.

the succession of bog vegetation onto a surface which once supported a well drained spruce forest.

Along this chronosequence of marine terraces, forest paludification is seen to accompany the development of spodosols. Iron cementation of B horizons, thickness of organic horizons, and hydraulic conductivity in these soils suggest a causative role for placic horizons in the paludification process. Other possible mechanisms of bog formation such as lake-infilling or the presence of impermeable parent material can be eliminated as alternative causes of paludification at this site. The spodosols responsible for paludification disappear in the anaerobic conditions occurring once the bog is established. Hence, little evidence for a pedogenic origin is available under existing bogs; a surface chronosequence seems necessary to document this process.

The role of soil development in peatland formation has been mentioned but seldom documented in the literature, and never previously assigned a major role in widespread paludification[9-11]. The data and model presented above suggest that pedogenically induced bog formation may be more common in South East Alaska and perhaps in other regions of the world than previously recognised.

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Behavioural access to short-term memory in bees

Randolf Menzel

Institut für Tierphysiologie, Freie Universität Berlin, Grunewaldstrasse 34, 1000 Berlin 41, FRG

Memory formation proceeds in temporal phases which differ in their effectiveness in controlling subsequent behaviour and in their susceptibility to amnestic treatments[1]. The initial phase of memory formation, frequently termed short-term memory, is generally considered a necessary precursor to long-term memory. However, the course of short-term memory differs widely between animal species and is dependent on experimental procedure. Information may even bypass the short-term phase en route to the long-term one[2,3]. Experiments reported here using honey bees in a behavioural learning situation suggest that the greatest significance of short-term memory is its function as a mode of memory storage which may be altered effectively by new and contradictory information. Freely flying honey bees were presented two colour alternatives and rewarded on first one and then the other in a reversal learning paradigm. Subsequent colour preference was dependent on the interval between the two trials. Several new features of short-term memory are described. It is concluded that a single mechanism of short- to long-term memory transfer cannot account for the observed bimodal interval dependent behaviour. Two mechanisms are proposed.

The experimental procedure resembled that used in previous studies in which freely flying bees were taught to associate food with a colour stimulus[2-5]. Several bees are trained to use a feeding station. A newly recruited bee is marked as the experimental animal and given three pretraining trails to a neutral grey background. Two coloured cards, blue and yellow, are then presented and spontaneous choice test used to establish that the bee has no colour preference. Actual training starts by rewarding the bee once on one of the two colours. Choice behaviour is tested after each reward. The experimental protocol is illustrated in Fig. 1. The first reward is on blue, the second on yellow. A reversed order of colour training gives the same results. Time interval between these trials is varied between 10 s and 10 min. This scheme tests the effects of information initiated by the second trial on that stored during the first. In addition, the effects of temporal separation of the two training trials are also elucidated. Previous results[2,3,5] suggested that the information capacity of short-term memory is both limited and time dependent. Thus variation of the inter-trial interval is a tool to describe short-term memory by a simple behavioural method. Another test after a second reversal learning trial gives information on the stability of these effects. Results are shown in Fig. 2.

Spontaneous choice activity was 50%. The results of learning and reversal learning in the long term are shown by arrows on the right ordinate of Fig. 2; reward duration was 60 s, and the interval between learning and reversal trials 10 min. The time course of interference between initial and reversal learning in the short term is examined by shortening the inter-trial interval. This is accomplished by decreasing reward duration to only a few seconds, making intervals as short as 10 s possible. The percent of choice behaviour has been shown to be independent of both duration and quantity of reward[2,6]. Accordingly, reward durations of 5 and 15 s were used. In both situations there was a dominant reduction in response to the initially rewarded blue colour mark for inter-trial intervals of 3 to 5 min. During this period information from the second trial, the first reversal trial

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on yellow, dominates the choice behaviour. At both greater and lesser inter-trial intervals choice behaviour was dominated by information from the initial trial on blue.

The results may be interpreted as follows. A learning trial initiates a short- term memory phase, which terminates 3–5 min later, and is followed by a consolidation phase. During the initial period of short-term memory and later with increasing consolidation new and contradictory information does not reach the memory store since the short-term memory and the consolidation process are already occupied by the information coming from the initial trial. In the transitional phase between short-term memory and consolidation, however, the information is more labile; it controls the choice behaviour less accurately[2,3,4] and more readily accepts new and contradictory information. This interpretation is in agreement with previous results[7] in which subjection of bees to electroconvulsive shock, cooling or N_2 and CO_2 narcosis disrupted memory only during the first few minutes after the learning trial. Since response levels in sessions A and B are similar and response functions from the first and second tests are parallel, the inter-trial interval must be the crucial parameter. No additional previously cryptic learning is expressed in the second test.

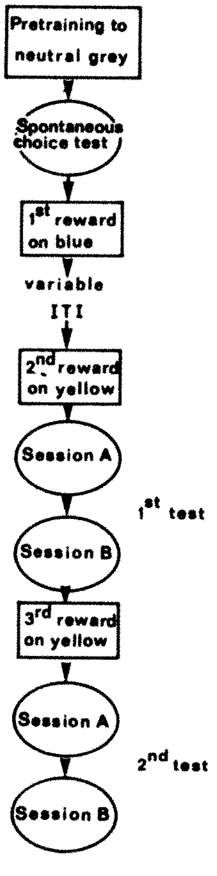

Fig. 1 Experimental scheme. Training sessions are shown in rectangles, and tests in elipses. Single, individually marked foraging bees (*Apis mellifera carnica*) are pretrained to grey cards and then tested for spontaneous choice between blue and yellow cards presented simultaneously. All cards are 7 cm in diameter. The first training reward is on blue, the second on yellow and the third on yellow. ITI (inter-trial interval) is defined as the period between the first and second training trials. Tests consist of two sessions, A and B. Each session lasts 4 min. Time interval between A and B is 3–6 min. Three classes of choice behaviour are monitored: (1) approaching the colourmarks, (2) touching colourmarks with antennae and/or legs, and (3) landing on colourmarks. Approach data are presented here. Touching and landing data show similar results.

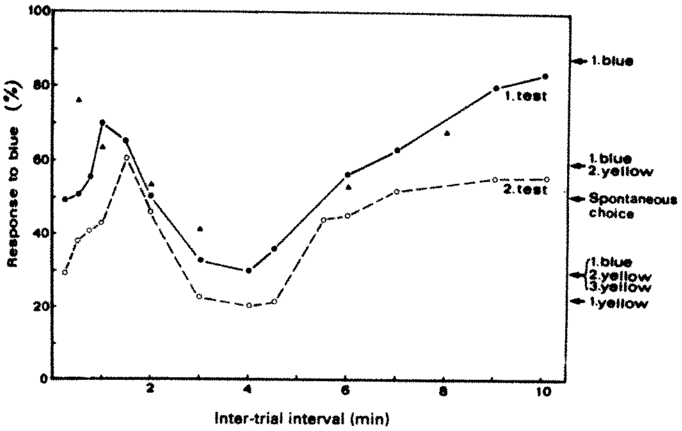

Fig. 2 Inter-trial interval and reversal learning. Results of sessions A and B are similar and, therefore, pooled. Circles are used to illustrate experiments with 5-s reward durations (no. of test animals 57; no. of choices 1,493); triangles represent experiments with 15-s reward duration (14 bees, 278 choices). Arrows on right ordinate: 1. blue, response level after one reward on blue (60-s reward duration); 1. blue 2. yellow, response level after a first reward on blue and a second on yellow (60-s reward duration); 1. blue 2. yellow 3. yellow, response level after a first reward on blue, a second on yellow and a third on yellow (60-s reward duration); 1. yellow: response level after one reward on yellow. In all cases averaged responses of five to eight bees are given. Differences of averaged responses of 15% or more are significantly different at the ≤0.01 level (χ^2 test, 2×2 table).

Our hypothesis does not account for the results obtained when the two training trials are presented within 1 min of each other. In this case, short-term memory should be strongest immediately after the trial[2,5,7]. However, for such short inter-trial intervals the bees switch to the second colour (see Fig. 2, first min of inter-trial interval). Furthermore, if bees are rewarded for 15 s (triangles in Fig. 2) there is not such an early reversal effect. We interpret these findings to indicate an additional mechanism which acts only during the period immediately following the initial trial and 'erases' the information just stored in the short-term memory by the action of new, contradictory information. This mechanism works only if contradictory information arrives in the period immediately following trial. Identical information in massed learning trials adds to each other and can even reach long-term memory directly[3].

Thus there are two temporal periods where reversal of learned behaviour is facilitated: The first ($t \leq 1$ min) is during the assimilation of the short-term phase, the second ($3 < t < 5$ min) is during the transition from short-term to consolidation phases. Information is eliminated most effectively if it has not yet been stored firmly in either the short-term or the long-term phase. The biological function of short-term memory is therefore not limited to that of a precursor for long-term memory; more importantly it is a mode of memory storage which allows for refinement by new incoming information.

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An unusual benzazocine elicits acetylcholine release in the isolated guinea pig ileum

R. J. Valentino, C. B. Smith & J. H. Woods

Department of Pharmacology, University of Michigan Medical School,
Ann Arbor, Michigan 48109

UM-1037 (3-allyl-1, 2, 3, 4, 5, 6-hexahydro-8-hydroxy-6-methyl-3-benzazocine), a benzazocine compound related structurally to the narcotic antagonists cyclazocine and the *N*-allyl analogue of cyclazocine, SKF-10,047, but lacking the 2,6-methano bridge, has been found to produce similar signs in normal rhesus monkeys to those characteristic of the narcotic abstinence syndrome[1]. Narcotic antagonists have been reported to produce contraction in the isolated guinea pig ileum[2,3]. However, only when the ileum has been continuously exposed to morphine or related drugs *in vitro* or has been isolated from animals made dependent on a narcotic, does it contract when an antagonist such as naloxone is added to the perfusion medium[4]. Thus, this preparation has been used as an *in vitro* model of the narcotic abstinence syndrome, and the contraction might be considered an 'abstinence sign'. In the present study, UM-1037 was found to produce a contraction of the guinea pig ileum isolated from normal animals similar to the contraction produced by naloxone in preparations exposed to morphine or related drugs. UM-1037, like naloxone, seems to act by releasing acetylcholine (ACh), because its actions were antagonised by atropine and tetrodotoxin. However, morphine, which is necessary for the naloxone-induced contraction, reversed the effect of UM-1037 on the ileum. Responses to UM-1037 were not altered in the presence of naloxone. Thus, the release of ACh which is produced by this unusual benzazocine in the ileum does not seem to be mediated by the opiate receptor.

The isolated guinea pig ileum was prepared as described by Paton[5]. Segments of ileum were suspended in a Krebs physiological buffer at 37 °C, saturated with 95% O_2–5% CO_2. The buffer contained NaCl, 118 mM; KCl, 4.75 mM; $CaCl_2.2H_2O$, 2.54 mM; $MgSO_4.7H_2O$, 1.19 mM; KH_2PO_4, 1.19 mM; glucose, 11 mM; $NaHCO_3$, 25 mM; hexamethonium Br, 0.07 mM; pyrillamine maleate, 0.125 μM. Resting tension on the tissue was 0.5 g. Tissues were equilibrated for 30–40 min with washes every 10 min. After the equilibration period, ACh, 5×10^{-6} M, was added to the bath to determine the maximum contraction of the tissue. Ten minutes after washing the ACh from the organ baths, cumulative concentration–response relationships were determined for UM-1037 by increasing the concentration of the drug by three-fold increments until a maximum response was obtained. After washout of UM-1037, ACh, 5×10^{-6} M, was added once again to determine whether the contractility of the preparation had changed.

Addition of UM-1037 to the organ bath produced a contraction of the isolated ileum (Figs 1, 2). The maximum contraction produced by UM-1037 was $53.5\pm8.1\%$ of the contraction produced by ACh, 5×10^{-6} M, and ED_{50} for UM-1037 was $5.33\pm0.97\times10^{-6}$ M ($n = 8$). The effect produced by UM-1037 in the isolated ileum not exposed to morphine-like drugs resembles that produced by naloxone in preparations exposed to such drugs[2].

As the contraction produced by naloxone is inhibited by atropine, 10^{-7} M, and tetrodotoxin, 2×10^{-7} M, it is thought to be caused by a release of ACh from postganglionic cholinergic nerve terminals in the myenteric plexus[6]. To determine whether the contraction produced by UM-1037 was due to a similar mechanism, we investigated the effects of atropine and tetrodotoxin on this response. Atropine, 10^{-8} M or 10^{-7} M, added to the organ bath 20 min before UM-1037, antagonised the effects of UM-1037 (Fig. 2). In the presence of atropine, the concentration–response curve for UM-1037 was shifted to the right, and the maximum response was decreased. Tetrodotoxin, 2×10^{-7} M, also blocked the response of the ileum to UM-1037 when added 10 min before UM-1037 (Fig. 2). Although this concentration of tetrodotoxin inhibited the electrically induced contraction of the ileum, it did not affect the response to ACh. The interactions with atropine and tetrodotoxin suggest that UM-1037, like naloxone, produces a contraction of the tissue by releasing a limited store of ACh from cholinergic neurones; this in turn acts on the muscarinic receptors of the intestinal muscle.

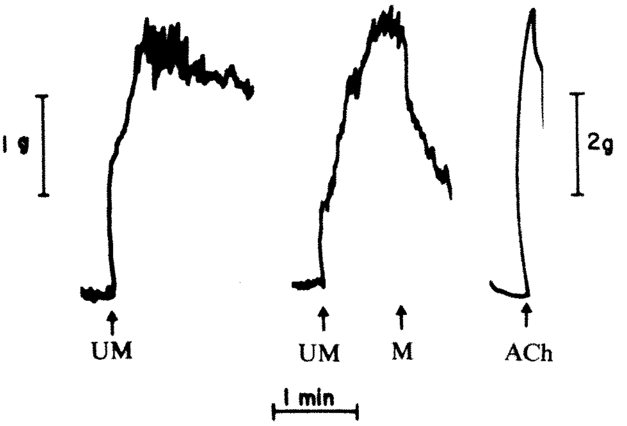

Fig. 1 Effects of UM-1037 on the isolated guinea pig ileum and antagonism by morphine. UM-1037, 3×10^{-5} M, was added to the organ bath at the time indicated by arrow UM. Morphine. 10^{-7} M, was added at arrow M. After washing the preparation, ACh, 5×10^{-6} M, a maximally effective concentration, was added to the organ bath at arrow ACh.

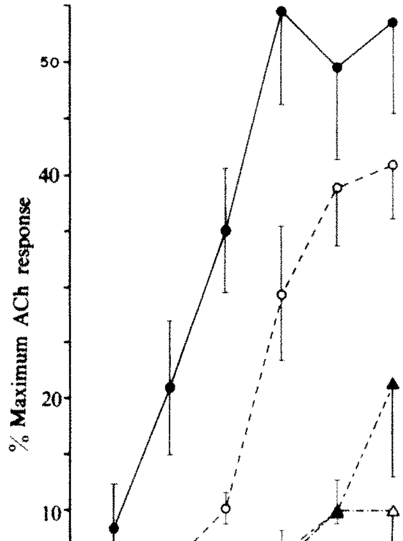

Fig. 2 Antagonism of UM-1037 by atropine and tetrodotoxin. Ordinate, per cent maximum contraction produced by ACh, 5×10^{-6} M. Abscissa, molar concentration. Concentration–response curves for UM-1037 in the guinea pig ileum are shown: eight control ilea (●——●); five ilea in the presence of atropine, 10^{-8} M, added to the bath 15 min before UM-1037 (○---○); five ilea in the presence of atropine, 10^{-7} M, added to the bath 15 min before UM-1037 (▲---▲); three ilea in the presence of tetrodotoxin, 2×10^{-7} M, added to the bath 10 min before UM-1037 (△—·—△). Mean values are given; vertical lines represent s.e.m.

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Morhpine-like agents inhibit the release of ACh from postganglionic cholinergic nerve terminals in the myenteric plexus of the guinea pig ileum[7,8]. This ability to inhibit ACh release is correlated with the ability to bind with specific receptors in the ileum[9]. To determine whether UM-1037 acts at specific opioid receptors, the interaction between UM-1037 and morphine was studied. Morphine, 10^{-7} M, reversed the contraction produced by a maximally effective concentration of UM-1037 (Fig. 1), and when added to the organ bath before UM-1037, it caused a parallel shift to the right in the UM-1037 concentration–response curve (Fig. 3). To ascertain whether muscarinic receptors in the ileum were affected by this concentration of morphine, the effect of carbachol was studied in the presence and absence of morphine. The ED_{50} values for carbachol alone, and when morphine, 10^{-7} M, had been added 5 min previously, were $1.15 \pm 0.09 \times 10^{-7}$ M ($n = 4$) and $1.37 \pm 0.38 \times 10^{-7}$ M ($n = 6$), respectively. Therefore, morphine reverses the effect of UM-1037 at a concentration which does not block muscarinic receptors. The antagonism of the effect of UM-1037 by morphine as well as by tetrodotoxin and atropine, further indicates that the contraction is due to release of ACh.

The interaction between morphine and UM-1037 may be due to competition at the same receptor site or to opposing effects mediated by interactions of the drugs at different receptor sites. Naloxone, a competitive narcotic antagonist, was used to elucidate this interaction further. Concentration–response relationships for UM-1037, determined 5 min after the addition of naloxone, 10^{-7} M, to the bath, did not differ from those for UM-1037 alone (Fig. 3). This suggests that naloxone and UM-1037 occupy different receptor sites. Naloxone, when added 5 min before morphine, 10^{-7} M, prevented the reversal of the effect of UM-1037 by morphine (Fig. 3). Because naloxone competes with morphine for opiate receptor-binding sites, it is probable that the antagonism of UM-1037 by morphine is mediated through the interaction of morphine with the opiate receptor to decrease ACh release in the guinea pig ileum. These results suggest that the interaction between UM-1037 and morphine does not occur by competition at a common receptor.

To determine whether UM-1037 is a narcotic antagonist, the effect of morphine was studied in the presence and absence of UM-1037. The preparation was coaxially stimulated at 0.1 Hz and supramaximal voltage for 1 h. Then cumulative concentration–response relationships were determined for morphine alone and repeated on other segments of tissue when UM-1037, 10^{-6} M, had been added 5 min previously. UM-1037 decreased the maximum inhibition produced by morphine (24.4% decrease), but did not produce a parallel shift to the right of the morphine concentration–response curve. The ED_{50} values for morphine in the presence and absence of UM-1037, 10^{-6} M, were $2.97 \pm 0.55 \times 10^{-8}$ M ($n = 5$) and $2.33 \pm 0.97 \times 10^{-8}$ M ($n = 5$), respectively. These results are consistent with the concept that morphine and UM-1037 do not compete for common receptors, and that UM-1037 decreases the maximum effect of morphine in the guinea pig ileum through an interaction with another site.

Prior exposure to a narcotic is a requirement for antagonist-precipitated abstinence in animals as well as for the antagonist-precipitated contraction of the isolated guinea pig ileum. The mechanism of narcotic antagonist-precipitated abstinence is generally thought to involve displacement of the narcotic from receptor sites.

In this study, the effects of UM-1037 on the isolated guinea pig ileum were studied. In this preparation, as in the monkey, a sign considered to be characteristic of narcotic abstinence was produced in the absence of narcotic exposure. The effects produced by this drug in the isolated guinea pig ileum resemble those produced by naloxone in preparations exposed to morphine in that release of acetylcholine mediates the contraction of the tissue in both cases. Unlike the naloxone-precipitated abstinence sign in the ileum, the effect produced by UM-1037 is antagonised by morphine.

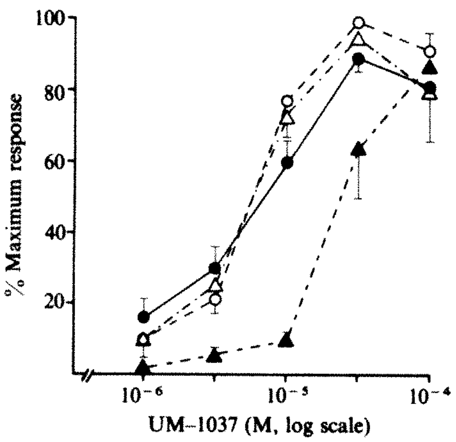

Fig. 3 Interaction between UM-1037, morphine and naloxone on the isolated guinea pig ileum. Ordinate, per cent maximum contraction. Abscissa, molar concentration. Concentration–response curves for UM-1037 in the guinea pig ileum are shown: eight control ilea (●——●); four ilea in the presence of morphine, 10^{-7} M, added to the bath 5 min before UM-1037 (▲---▲); three ilea in the presence of naloxone, 10^{-7} M, added to the bath 5 min before UM-1037 (○---○); four ilea in the presence of both naloxone, 10^{-7} M, added to the bath 10 min before UM-1037 and morphine, 10^{-7} M, added to the bath 5 min after naloxone and 5 min before UM-1037 (△—·—△). Mean values are given; vertical lines represent s.e.m.

A syndrome resembling the narcotic abstinence syndrome has been described in normal rats after the administration of phosphodiesterase inhibitors[11]. This 'quasi-abstinence syndrome' is antagonised by narcotics and potentiated by naloxone. However, UM-1037 does not act like a phosphodiesterase inhibitor. Although morphine antagonises the effect of UM-1037 in the isolated guinea-pig ileum, naloxone does not alter the response to UM-1037. Furthermore, theophylline, a methylxanthine which is a phosphodiesterase inhibitor, does not produce a contraction of the isolated guinea pig ileum, although the methylxanthines do enhance the electrically induced contraction of the ileum[12]. At high concentrations theophylline inhibits the twitch of the stimulated preparation[12]. UM-1037 produces a contraction of the isolated ileum and does not inhibit the twitch at any concentration. Therefore, UM-1037 and phosphodiesterase inhibitors do not seem to share a common mechanism of action in this preparation.

Like UM-1037, nicotine and 5-hydroxytryptamine cause a release of ACh from cholinergic neurones in the isolated guinea pig ileum[12]. Preliminary studies indicate that the actions of UM-1037 on this preparation are not mediated by either nicotinic or tryptaminergic receptors[13,14].

Although the present study suggests that the precipitation of the abstinence syndrome by narcotic antagonists in morphine-dependent ilea and the abstinence-like syndrome produced by UM-1037 in non-dependent ilea involve different receptor sites, it is possible that common mediators or neural pathways are involved in both drug effects. UM-1037 and related compounds may be important in elucidating the mechanism underlying the narcotic abstinence syndrome.

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Curare has a voltage-dependent blocking action on the glutamate synapse

Daisuke Yamamoto & Hiroshi Washio

Laboratory of Neurophysiology, Mitsubishi-Kasei Institute of Life Sciences, Machida, Tokyo 194, Japan

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The skeletal muscle of members of Orthoptera and Diptera receives an innervation which is probably glutaminergic[1–3]. Recent study of the ventral muscle fibres in the larvae of the beetle, *Tenebrio molitor*, has revealed that the transmitter action can be mimicked by the iontophoretic application of L-glutamate to the junctional sites at which the extracellular excitatory postsynaptic potentials (e.p.s.ps) could be recorded (D.Y. and H.W., unpublished observation). Contrary to the evidence favouring glutamate as a transmitter of junctional excitation in insects, some investigators have found that curare (+)tubocrarine, TC), a classic acetylcholine (ACh) antagonist, suppresses the neurally evoked muscle potentials in the fly *Sarcophaga*[4,5], and *Tenebrio*[6]. Here, we have analysed the action of curare on the neuromuscular junction of *Tenebrio* larvae and found that curare blocked the glutaminergic transmission by antagonising the transmitter at the postsynaptic site.

The dissection used has been described previously[7]. The ventral muscle fibres were penetrated by two microelectrodes, one for recording and another for passing current, filled with 3 M KCl and 2 M K citrate, respectively. When necessary, the muscle fibres were voltage clamped using a two-microelectrode method[8]. For focal extracellular recording, electrodes were filled with 3 M NaCl. Although the signals recorded extracellularly could be resolved into single traces on the oscilloscope, a minicomputer (Sanei signal processor 7T07A) was used to obtain a signal averaging so as to improve the signal-to-noise ratio. The iontophoretic electrodes were filled with 1 M solution of monosodium L-glutamate (*p*H 8.0). The e.p.s.ps were evoked by stimulating the innervating nerve close to the segmental ganglion. All experiments were carried out in a low Ca^{2+} saline solution to any minimise movement artefacts accompanying an action potential. The standard bathing saline solution had the following composition (mM): NaCl, 70; KCl, 30; $CaCl_2$, 0.8; $MgCl_2$, 14.2; glucose, 445; HEPES, 5. The *p*H of the solution was adjusted to 7.2 with NaOH.

Curare, when applied to the bathing solution at concentrations higher than 5×10^{-4} M, reversibly depressed the e.p.s.p. amplitude without affecting either the input membrane resistance or the resting potential of the muscle fibre. Simultaneous focal recordings have revealed that the postsynaptic current was substantially depressed but that the presynaptic impulse and the synaptic delay remained essentially unchanged (Fig. 1). The quantum content estimated by the failure method[9] was unaffected by the application of curare (Table 1). These facts indicated that the site of action of curare was on the postsynaptic membrane. The observations do not necessarily mean that the presynaptic terminal of *Tenebrio* neuromuscular junction lacks the ACh receptors reported to be present on the locust junction[10], because curare failed to affect transmitter release even in the locust system unless it was applied simultaneously with ACh[10,11].

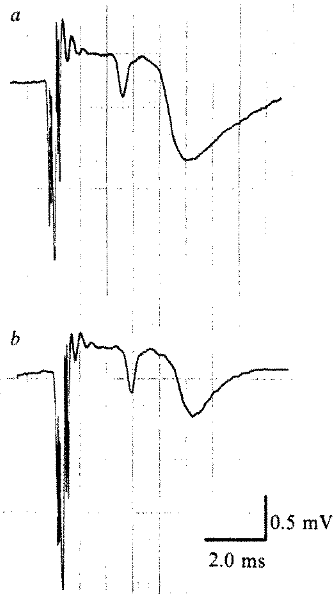

Fig. 1 The nerve terminal action potential and the e.p.s.p. recorded extracellularly from the neuromuscular junction. Records were taken before (*a*) and 5 min after (*b*) bath perfusion of 7.5×10^{-4} M curare. Signals were averaged over 99 trials.

To characterise the curare-induced suppression further, we examined the action of curare on the glutamate potential. We found that the amplitude of the glutamate potential decreased and the time to peak was markedly prolonged after treatment with 5×10^{-4} M curare. One possible explanation for the prolonged glutamate potentials in TC solution is that the L-glutamate molecules ejected must diffuse over a greater distance to reach the sensitive receptors than would be required in normal conditions because some accessible receptors which are distributed on the fibre surface may become unavailable through the action of TC. Both effects of curare on the glutamate potential were fully reversible. Curare also produced a reduction in the apparent maximum of the dose–response curve for glutamate-induced depolarisation (Fig. 2). The non-competitive

Fig. 2 Effect of curare on the dose–response curve to glutamate recorded from a single mealworm muscle fibre: Results are of glutamate-evoked membrane depolarisation measured in control solution (●) and in 7.5×10^{-4} M curare (○).

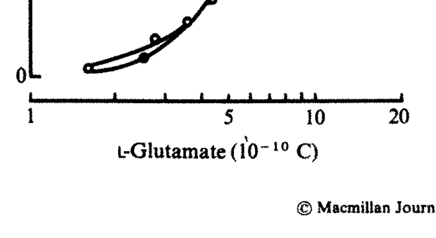

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Table 1 Effect of curare on quantum content

Expt	Concentration (mM)	Before TC	During TC	Quantum content ratio (during/before)
1	0.75	2.15	2.03	0.94
2	0.75	1.74	1.56	0.90
3	0.5	1.03	0.90	0.87
4	0.5	1.41	1.82	1.29
Mean and s.e.				1.00 ± 0.098

Quantum content was calculated from the number of failures of extracellular e.p.s.ps. The number of stimuli ranged from 198 to 396; the stimulation rate was 5 Hz.

antagonism has been taken as evidence that the drug blocks ionic channels mediated by the transmitter rather than acting on the receptor itself[12]. Furthermore, there is additional evidence supporting the notion that curare is acting on the glutamate channel. Thus, in contrast to its normal behaviour, the amplitude of the glutamate current under voltage clamp does not increase with hyperpolarisation, but may actually be reduced (Fig. 3). At cholinergic synapses in *Aplysia* neurones[13] and frog muscle[14], curare has been shown to produce a negative slope in the membrane potential–ACh current relationship with hyperpolarisation, but the amplitude of the excitatory postsynaptic current (e.p.s.c.) increased linearly with hyperpolarisation even in the presence of curare ('Manalis effect' (ref. 15)). Contrary to results in these cholinergic synapses, curare influenced the membrane potential–e.p.s.c. relationship in a similar manner to that observed in the iontophoretically induced responses in the present system. Representative families of e.p.s.cs before and during application of curare are shown in Fig. 4. In this respect, the action of curare on the glutamate-mediated junction resembles that of local anaesthetics on the cholinergic synapse. For example, procaine produces a non-linearity of the membrane potential–e.p.s.c. amplitude relationship in the frog neuromuscular junction[16], suggesting that the drug becomes more effectively attached to its binding site in the activated receptor–mediator complex during hyperpolarisation. As in the case of procaine blockade of the cholinergic synapse, curare might combine with the receptor–transmitter complex, thereby 'plugging' the open channel itself[15,17,18]. Alternatively, the present results showing an effect of curare on the e.p.s.c. as well as on the glutamate current may favour a model[19] in which local anaesthetics can bind to the receptors before ACh does.

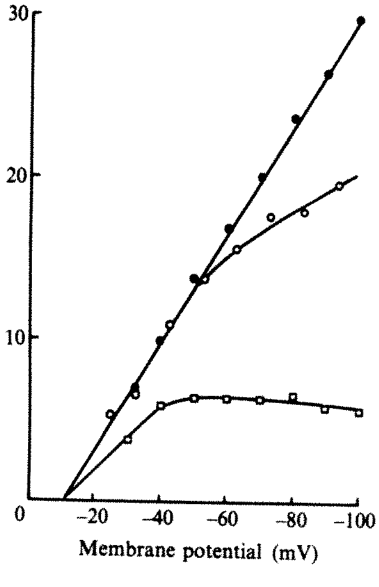

Fig. 3 Voltage dependence of the amplitude of the glutamate current measured before (●) and 5 min after (□) perfusion of 7.5×10^{-4} M curare and 15 min after washing with drug-free saline (○). The recovery after washing is incomplete in this fibre.

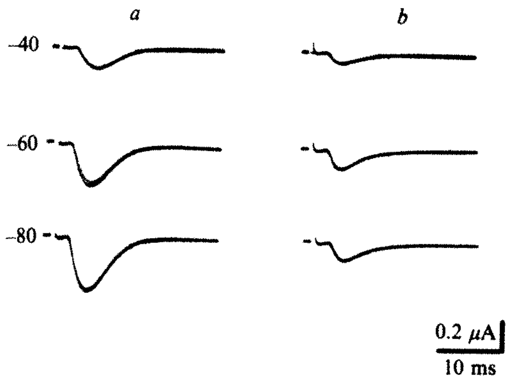

Fig. 4 Excitatory postsynaptic currents recorded at three different holding potentials (indicated at the left side of the each trace) before (*a*) and during (*b*) application of 7.5×10^{-4} M curare. Three successive e.p.s.cs evoked by repetitive stimulations at 0.5 Hz were superimposed on each record. Downward deflections indicate inward currents.

The blockade of non-cholinergic transmission by curare has already been reported in *Aplysia* neurones[20,21]—depolarisations induced by dopamine and serotonin as well as Cl-dependent hyperpolarisation caused by serotonin were suppressed by curare. In addition, K^+-dependent hyperpolarisations induced not only by dopamine and serotonin but also by ACh were completely insensitive to curare. Therefore, it seems possible that curare acted on the ionic channels rather than on the receptors in *Aplysia* neurones.

The present experiments demonstrate that the site of action of curare in *Tenebrio* ventral muscle is on the subsynaptic membrane. However, there is no evidence that curare has similar effects on synapses in other *Tenebrio* muscles. Reports that curare is inactive at synapses on some other insect muscles, such as *Periplaneta*[22] and *Schistocerca*[23], probably reflect a species difference. The results are against the explanation that curare can indirectly depress the muscular electrical potentials by blocking conduction in the motor nerves[24–26]. The study also suggests that results using curare as a pharmacological indicator of cholinergic involvement should be interpreted with caution.

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Recycling of noradrenergic storage vesicles of isolated rat vas deferens

Arun R. Wakade

Department of Pharmacology, State University of New York,
Downstate Medical Center, 450 Clarkson Avenue, Brooklyn,
New York 11203

Del Castillo and Katz established the theory of quantal release of acetylcholine from the neuromuscular junction[1]. The concept was expanded by showing that quanta of transmitter was packaged in, and released from, storage vesicles of the nerve endings. Noradrenaline (NA) is also believed to be stored in, and released from, storage vesicles of the sympathetic nerves. The requirement of Ca for NA release[2] and concomitant secretion of the vesicular enzyme, dopamine-β-hydroxylase[3], on stimulation lends strong evidence to the theory of exocytosis. However, it remains unclear whether storage vesicles after fusing with neuronal membrane to release NA into cleft, are once again reused for further storage and release of NA, or are discarded by the nerve endings. I have investigated this question by making use of the fact that tetraethylammonium (TEA) causes profound facilitation of the overflow of ^{3}H-NA when the sympathetic nerves of the isolated vas deferens are electrically excited[4], and that this excess release is accompanied by a significant reduction in tissue NA content[5]. The principal advantage of the method is that the reduction in tissue NA content is effected exclusively by the exocytotic release of NA from the nerve terminals and easy washout of TEA allows one to study uptake of exogenous NA by the tissue. The results presented here support the hypothesis that NA storage vesicles are recycled.

Male rats weighing 300–400 g were killed by a blow on the head, and the vasa deferentia were removed. Vasa deferentia were placed in 1 ml Krebs solution gassed with 95% O_2–5% CO_2, and incubated at 37 °C for 30 min, and then incubated for 15 min with 0.35 μM ^{3}H-labelled (±)NA (specific activity, 13 Ci mmol^{-1}, Amersham Searle). Following termination of incubation, the tissues were washed three times with Krebs solution over a 30-min period, at the end of which tissues were removed to study either NA content and ^{3}H-NA accumulation or the overflow of ^{3}H-NA upon transmural stimulation as described previously[6]. In the case where repetitive stimulation was used for longer periods, a train of 150 impulses at 5 Hz was delivered every 30 s of 1 min for varying periods of time (80 V, 1-ms duration) from a Grass stimulator, Model S-88. In the case where overflow of ^{3}H-NA was studied before and after TEA and repeated stimulation, the vas deferens was stimulated by a train of 900 impulses at 30 Hz. To obtain net overflow of ^{3}H-NA released by stimulation, the amount of radioactivity spontaneously released within the immediately preceding equivalent time period was subtracted from the total amount released during the stimulation period[6]. Overflow of ^{3}H-NA is expressed as a fraction of the total ^{3}H-NA content of the tissue at the onset of stimulation. Endogenous NA content of the vas deferens was analysed fluorometrically[7] as described earlier[8]. The same method was used to separate ^{3}H-NA and ^{3}H-catechol metabolites from the tissue bathing medium as previously described[6].

As TEA enhances stimulation-induced overflow of radioactivity in the ^{3}H-NA-labelled rat vas deferens by about 20-fold at 5 Hz, as compared to a four- to five-fold increase with phenoxybenzamine[4], TEA was used to study its effect on tissue NA content at rest and during nerve activity. As shown in Table 1 the NA content of right vas deferens exposed to TEA was comparable to that of the contralateral control tissue. Intermittent transmural stimulation of the right vas deferens caused no significant change in the NA content. However, transmural stimulation in the presence of TEA significantly lowered tissue NA content. Figure 1 shows the combined effect of TEA and transmural stimulation on tissue NA content for different time periods. A significant reduction in NA content (27±4%, $P<0.01$) was found after 30 min and the degree of reduction increased with the exposure to TEA and transmural stimulation. A maximum reduction of 87% occurred after 4 h.

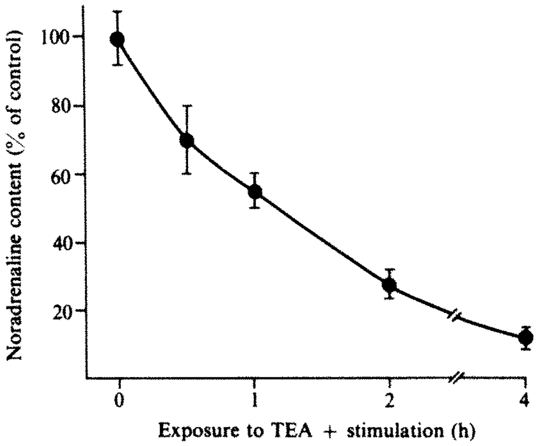

Fig. 1 NA content of the vas deferens various times after exposure to TEA and transmural stimulation. The right vas deferens was exposed to 10 mM TEA plus transmural stimulation (5 Hz, 30 s min^{-1}) for different time periods. The NA content of the treated tissue is expressed as a percentage of that found in contralateral control tissue (11.6±0.1 μg g^{-1}, $n=20$) kept in 10 mM TEA–Krebs solution for a comparable time. Each point, except the 0-h point, represents a mean of 5 observations. Vertical lines show s.e.m.

NA is equally distributed throughout the vas deferens[8]. Therefore, during the course of an experiment the tissue can be cut in several portions, each portion initially having comparable amounts of NA (or ^{3}H-NA). Initial NA and ^{3}H-NA contents present in a small portion of the vas deferens were 13.01±0.75 μg g^{-1} and 4,520±412 c.p.m. mg^{-1}, respectively (Fig. 2).

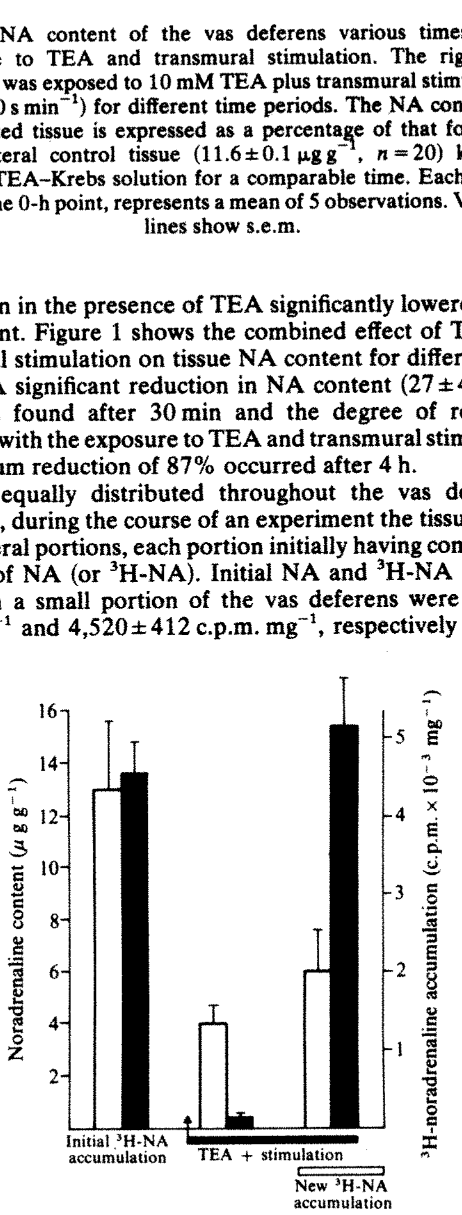

Fig. 2 Depletion and retention of ^{3}H-NA before and after TEA and transmural stimulation for 2 h. NA content (open columns) and ^{3}H-NA accumulation (solid columns) are shown in control tissue (first paired columns), in the same tissue after exposure to TEA plus stimulation for 2 h (Fig. 1) (middle paired columns), and in the same tissue after exposure to TEA plus stimulation for 2 h, and then incubation with ^{3}H-NA (last paired columns). For details see text. Each column represents a mean of 5 experiments. Vertical lines show s.e.m.

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Table 1 Effects of TEA and transmural stimulation, separately and together, on NA content of the vas deferens

Experimental conditions	No. of experiments	NA content Control, left vas deferens ($\mu g\ g^{-1}$)	 Experimental, right vas deferens	% Of control
TEA (10 mM, for 2 h)	5	10.06 ± 1.40	12.08 ± 1.70	120 ± 13 (NS)
Transmural stimulation (5 Hz, 30 s min^{-1}, for 2 h)	7	12.08 ± 1.70	12.80 ± 1.0	106 ± 9 (NS)
TEA + transmural stimulation	7	11.40 ± 0.8	2.90 ± 0.40	25 ± 6*

After 30 min equilibration of both vasa deferentia in Krebs solution, right vasa deferentia were exposed to either TEA, transmural stimulation, or TEA plus transmural stimulation for a total period of 2 h. Left vasa deferentia remained in Krebs solution (first 2 groups of experiments) or in 10 mM TEA (the third group) for 2 h to serve as controls. NS, not significantly different from the control.

* $P < 0.001$.

The remaining tissue was subjected to the combined treatment of TEA and transmural stimulation for 2 h. TEA was washed out for 15 min and the tissue was cut into two portions; one was again incubated with ^{3}H-NA and subsequently washed, while the other remained in Krebs solution. TEA and stimulation lowered NA content by about 70%, and the ^{3}H-NA level was 3% of the initial control. Such depleted tissue, when re-exposed to ^{3}H-NA, took up and retained large quantities of ^{3}H-NA. The new accumulation of ^{3}H-NA was comparable to the initial accumulation by the control tissues not subjected to TEA and stimulation.

As maximum depletion of NA occurred at 4 h after treatment with TEA and stimulation (Fig. 1) the accumulation of ^{3}H-NA in such severely depleted tissues was investigated. NA content was lowered from a control value of 9.54 ± 0.79 to $1.24 \pm 0.3\ \mu g\ g^{-1}$, and ^{3}H-NA was undetectable after TEA plus stimulation for 4 h. On re-exposure to ^{3}H-NA, such depleted tissue retained ^{3}H-NA in amounts identical to those found in control tissue before depletion ($4{,}190 \pm 396$ against $3{,}826 \pm 321$ c.p.m. mg^{-1}).

Cocaine was used to determine the sites of new accumulation of ^{3}H-NA by the depleted vas deferens. In the control tissue, 3.6 μM cocaine reduced ^{3}H-NA accumulation by 74% ($3{,}238 \pm 605$ against 862 ± 190 c.p.m. mg^{-1}, $n = 3$). Cocaine had a similar effect on ^{3}H-NA accumulation in tissue initially depleted by TEA and stimulation ($3{,}260 \pm 388$ against 754 ± 173 c.p.m. mg^{-1}, $n = 3$).

Figure 3 shows the results of experiments in which overflow of newly acquired ^{3}H-NA was studied in tissues previously subjected to TEA and stimulation. Following initial ^{3}H-NA incubation and its washout, the overflow of ^{3}H-NA induced by stimulation (30 Hz) averaged 17.9 ± 2.2 ($\times 10^{-4}$). TEA and transmural stimulation lowered ^{3}H-NA levels to such an extent that no overflow from such tissues could be measured. However, on reloading of the tissue with ^{3}H-NA, overflow induced at 30 Hz was almost identical to the initial overflow 18.7 ± 2.6 ($\times 10^{-4}$). The new overflow was depressed by over 90% in the absence of external Ca (Fig. 3*b*). Substitution of 2.5 mM Ca restored the new overflow to almost 75% of the initial control value.

As stimulation alone does not alter tissue NA content over a 2-h period, the amounts released through this method may be replenished by either the synthesis of new NA or uptake of released NA, or both of these mechanisms. With TEA and stimulation NA release must be so great that even accelerated synthesis together with reuptake cannot keep up with the release, resulting in a reduction in tissue NA content. Ca is needed for the TEA-induced facilitation of release during nerve stimulation. Therefore, the depletion of NA content of the isolated vas deferens must be a result of exhaustive exocytotic release of NA. The participation of newly synthesised storage vesicles can be totally excluded in this study, as this cannot be done in the case of the adrenal gland or sympathetic organ *in vivo*, because the hypogasting plexus innervating the vas deferens[8] is not present in the preparation. Although certain procedures reduce the NA content of the isolated tissues, these did not involve the exocytotic release of NA. It was shown that Na deprivation results in reduction in NA content of cat spleen slices[9] and the rabbit heart[10] without requiring external Ca. However, intermittent stimulation of splenic nerves of the dog in the presence of phentolamine caused significant loss of splenic NA content[11].

As it was shown that the amounts of ^{3}H-NA retained by previously depleted vas deferens were almost identical to those retained by control tissues and as cocaine, a well-known inhibitor of neuronal uptake of NA[12], blocked initial and new accumulation of ^{3}H-NA to the same extent, it is believed that the new accumulation occurs predominantly in the sympathetic nerves. If NA taken up by the neuronal membrane is not removed by the storage vesicles, then it is rapidly deaminated by intraneuronal monoamine oxidase[13]. In the present work it was found that even 30 min after washout comparable amounts of ^{3}H-NA were retained by normal and depleted tissues. Therefore, it is assumed that ^{3}H-NA taken up by depleted tissue is retained by the storage vesicles of the sympathetic nerve ending. Strong proof for the idea that the newly taken up ^{3}H-NA is accrued to the storage vesicles is that stimulation releases newly acquired ^{3}H-NA. It is known that if ^{3}H-NA is not present in the storage vesicles, but is accumulated in extravesicular sites in the sympathetic nerves, it is not avavilable for release by electrical stimulation[14]. The fact that newly acquired ^{3}H-NA can be

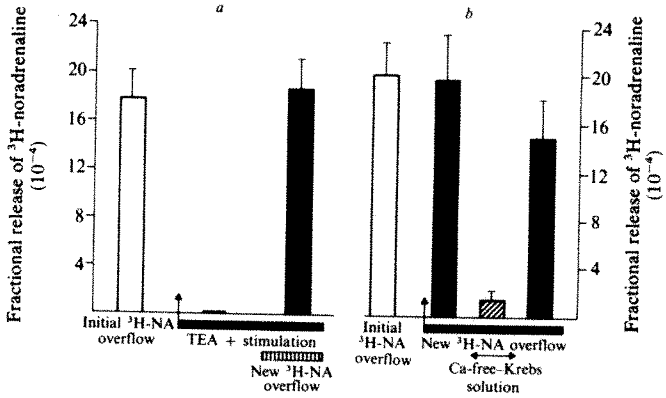

Fig. 3 Overflow of ^{3}H-NA before and after TEA plus transmural stimulation for 2 h. Overflow of ^{3}H-NA was induced by stimulating (30 Hz for 30 s) the initially labelled vas deferens (open column). Overflow of ^{3}H-NE is expressed as fractional release which represents the ratio of the overflow of ^{3}H-NA to that in the tissue at the beginning of the stimulation. The solid column represents the overflow elicited from relabelled tissues subjected to 10 mM TEA plus transmural stimulation in Krebs solution, as described above, then in Ca-free Krebs solution (hatched column), and finally in Krebs solution. *a*, Each column represents a mean of 8 experiments and in *b* represents a mean of 4 experiments. Vertical lines show s.e.m.

released in response to nerve stimulation, and that the new release is Ca dependent, would indicate that the release occurs by exocytosis.

The main question now is whether the same storage vesicles which participate in the storage and release of NA are responsible for the accumulation and subsequent release of ^{3}H-NA. As the maximum depletion of NA stores was 87% after TEA and stimulation, it may be that remaining storage vesicles which did not participate in exocytosis could take over the function of storage and release of the total original population of vesicles. Another possibility is that storage vesicles previously involved in exocytosis can be reused by sympathetic nerve terminals for the purpose of recapturing and releasing new NA.

It has been suggested that the membrane of the large noradrenergic vesicle in nerve terminals may be retrieved after exocytosis in the form of small vesicles. The latter, then, might be the precursors of the functioning small vesicles[15]. In support of such an idea, it has been shown that newly formed small storage vesicles after nerve activity contain significant amounts of dopamine β-hydroxylase, and these vesicles are capable of storing ^{3}H-NA[11]. An alternative suggestion is that after a vesicle fuses with the nerve membrane it becomes nonfunctional and is destroyed by the nerve[16]. Based on the experiments reported here, I feel that noradrenergic storage vesicles of the rat vas deferens can be reused more than once by nerve terminals. However, the lack of recovery of endogenous NA, following its depletion by exhaustive exocytosis, may be an indication that used vesicles lose their ability to synthesise NA and are only capable of storing preformed NA.

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A new neuronal marker identified by phosphorylcholine-binding myeloma proteins

Elisabeth L. Hooghe-Peters*

Section on Electron Microscopy, Infectious Diseases Branch, National Institute for Neurological and Communicative Diseases and Stroke,

Betty J. Fowlkes

Laboratory of Microbial Immunity, National Institute of Allergy and Infectious Diseases

Robert J. Hooghe†

Laboratory of Cell Biology, National Cancer Institute,
National Institutes of Health, Bethesda, Maryland 20014

The difficulty of working with the intact brain *in vivo* has led to the increasing use of nerve cell cultures in neurobiology. However, dissociated cells cannot be unambiguously identified by morphological criteria before the third week in culture, for it is not until then that the basic morphology and size of neurones become stable so that these and other cell types can be easily distinguished[1]. However, cultured neurones can be identified by various cytochemical techniques based on (1) the detection of neurotransmitters or receptors for transmitters[2–4], (2) the presence of the Thy 1 antigen and the receptor for tetanus toxin, which are present on the membrane of most neurones[5], and (3) the presence in neurones of neurone-specific enolase (NSE), a cytoplasmic enzyme, which can only be identified on fixed specimens[6,7]. Furthermore, other cell types in culture can also be specifically labelled. For instance, antisera to galactocerebroside bind selectively to oligodendrocytes[8], and antibodies to a neural tumour bind selectively to Schwann cells[5,8,9]. We report here the selective interaction of phosphorylcholine-binding myeloma proteins (PC-BMP)[10] with mouse neurones in culture and in suspension. Phosphorylcholine (PC) is found as part of lecithin and sphingomyelin molecules in variable amounts in eukaryotic and prokaryotic membranes, including plasma membranes[11–13].

Dissociated cells from the mouse (C57/B6) nervous system were cultured as previously described[1]. Briefly, spinal cord, dorsal root ganglia or both, were dissected from 12–13-d embryos and cleaned of any adhering tissue. Excised tissue was cut into small pieces, trypsinised (0.25% in Eagle's minimal essential medium (MEM)) and further mechanically dissociated. Cells were seeded (5×10^5 cells per coverslip) on to 12-mm glass coverslips in 24-well plates (Cluster 24, Costar) coated with 0.05% collagen (Calbiochem) in 0.1% acetic acid. Cells were grown under 90% air and 10% CO_2 in bicarbonate-buffered MEM supplemented with 600 mg glucose per 100 ml, 10% heat-inactivated horse serum and, until the cultures reached confluency, 10% fetal calf serum. When confluent (at about 5–7 d), they were exposed for 2 d to 5-fluorodeoxyuridine (Sigma, 1.2 mg per 100 ml) and uridine (3 mg per 100 ml). This treatment, which prevents overgrowth of dividing non-neuronal cells, was repeated 2 weeks later if thought necessary. The medium was changed three times per week. Most experiments were carried out 4 weeks after plating, when the majority of cells could be identified by phase contrast microscopy on morphological criteria. These cultures were fixed with paraformaldehyde (1% for 5 min). Some experiments were carried out on freshly dissociated living cells in suspension.

The myeloma proteins used in these experiments included the following: TEPC 15; MOPC 167 and McPC 603, PC-BMP; XRPC 25, a dinitrophenol-BMP; EPC 109 and UPC 61, inulin-BMPs; XRPC 24, a $\beta(1-6)$ galactan-BMP; and McPC 870, a lipopolysaccharide-BMP which also weakly binds PC[10]. All proteins were IgAκ and were purified by affinity chromatography, with the exception of McPC 870, which was isolated by ion-exchange chromatography followed by gel filtration. Goat anti-mouse IgA antibodies were raised, purified by affinity chromatography and tested as described elsewhere[14,15]. The antiserum was passed over a mixed IgG (MOPC 195, MOPC 70 A, MOPC 173 myelomas)–Sepharose column and then eluted from an IgA (TEPC 15)–Sepharose column. The purified antibodies (GAMα) were coupled to fluorescein by reaction with fluorescein isothiocyanate (FITC)[14] and a fluorescein : protein molar ratio of 4.5 was obtained. Rhodamine-coupled tetanus toxin (RTT) was prepared as described in ref. 16.

Fixed cells were exposed to MPs at concentrations up to 1 mg ml^{-1} for 1 h at room temperature, washed in phosphate-buffered saline (PBS) and incubated in GaMα (0.5 mg ml^{-1}) for 1 h at room temperature. For double-labelling experiments RTT, PC-BMP and GaMα were applied successively, each for 1 h at room temperature. Cells were mounted on glass slides for

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[*] Present address: Institute of Histology and Embryology, University of Geneva Medical School, 1211 Geneva 4, Switzerland.

[†] Present address: Department of Pathology, Sciences II, 30, quai Ernest Ansermet, 1211 Geneva 4, Switzerland.

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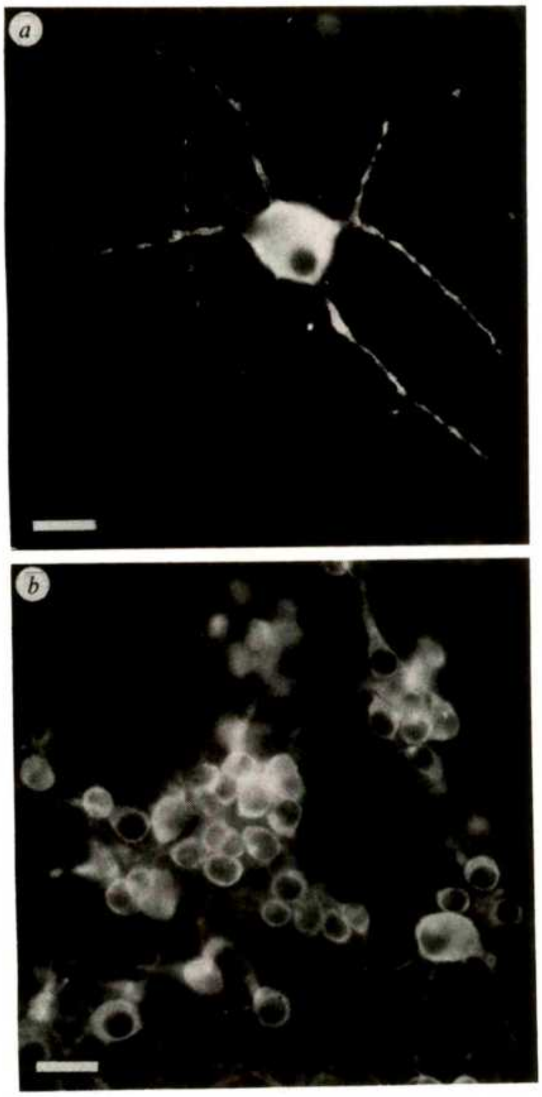

Fig. 1 Immunofluorescence of fixed mouse spinal cord and dorsal root ganglia cells. Four weeks after seeding, cells attached to coverslips were washed with serum-free MEM, fixed in 1% paraformaldehyde in PBS for 5 min at room temperature and washed extensively with PBS. The fixed cells were incubated with affinity-purified PC-BMP, in this case MOPC 167 (1 mg ml^{-1}), for 60 min at room temperature. After washing with PBS, they were incubated with fluorescein isothiocyanate-labelled goat anti-mouse α-chain antibodies (0.5 mg ml^{-1}) for 30 min at room temperature and washed with PBS. The coverslips were mounted on slides with 50% glycerol in PBS and were examined with a Zeiss microscope equipped with epi-illumination. *a*, Only one cell is labelled. It has the typical morphology of a neurone from dorsal root ganglia. The underlying and surrounding cells are negative. Scale bar, 33 μm. *b*, A cluster of smaller spinal cord neurones and one probable dorsal root ganglionic cell are shown. Only neurones are labelled. Cell processes are also labelled, although they are not visible in this picture, where the plane of focus falls on the cell bodies. Scale bar, 40 μm.

observation on a Zeiss microscope equipped with fluorescein and rhodamine optics. NSE was detected on parallel mature cultures as previously described[7]. Double-labelling with PC-BMPs and antibodies to NSE was not possible because of the severe conditions required for the detection of NSE. Live cells were stained for surface antigens immediately after dissociation. A pellet of 10^6 cells was resuspended in 50 μl of MP (1 mg ml^{-1} in PBS) for 20 min at 4°C. Cells were then washed twice, incubated with FITC GaMα (0.5 mg ml^{-1}), washed again, re-suspended in a minimal volume and sealed with a coverslip on a microscope slide.

On mature cultures, all cells with neuronal morphology (that is, phase bright somata, distinct nuclei and process extensions[1]) were stained with PC-BMP MOPC 167 whereas flat background cells (presumably fibroblasts and glial cells) were non-reactive (Fig. 1). A cell type indistinguishable from that stained by MOPC 167 was also stained by antiserum to NSE (a cytoplasmic marker) (Fig. 2) and by tetanus toxin. The latter was used in a double-labelling experiment and all the cells which showed positive fluorescein staining by PC-BMP and FITC GaMα were also stained by tetanus toxin conjugated to rhodamine. However, labelled tetanus toxin also stained some background cells. Identical results were obtained when PC-BMPs other than MOPC 167 were used. Thus, TEPC 15 and McPC 603 behaved exactly like MOPC 167. MP McPC 870 also stained neurones in the same way, although it binds PC with much lower affinity than do the other PC-BMPs[10]. The binding of PC-BMPs to neurones

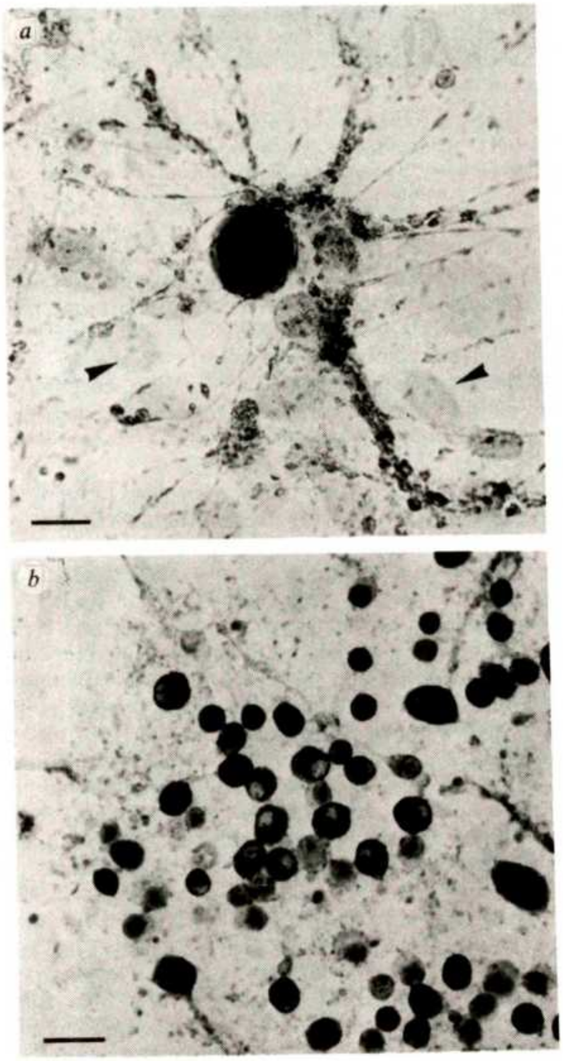

Fig. 2 Immunoperoxidase staining of mouse spinal cord and dorsal root ganglia cells after 4 weeks in culture. Cells were fixed in 4% paraformaldehyde, 1% glutaraldehyde, 0.2% picric acid and 1.5% sucrose in 0.1 M sodium acetate buffer, *p*H 6.0, for 1 h. The fixed cells were washed in 0.05 M Tris-HCl and 0.15 M NaCl, *p*H 6.0, then treated with 0.25% Triton X-100 in the same buffer for 10 min, and washed again. The monolayers were incubated with rabbit antiserum to neurone-specific enolase (1 : 800 in PBS) then with protein A–peroxidase (0.05 mg ml^{-1}) and finally reacted with diaminobenzidine and H_2O_2, as previously described[7]. Monolayers were counterstained with cresyl violet, dehydrated and mounted. Neurones show positive staining for NSE in their soma as well as their processes. In addition to the labelled neurones, the nuclei of several unlabelled cells can be seen (arrowheads). Scale bars, 33 μm(*a*) and 40 μm (*b*).

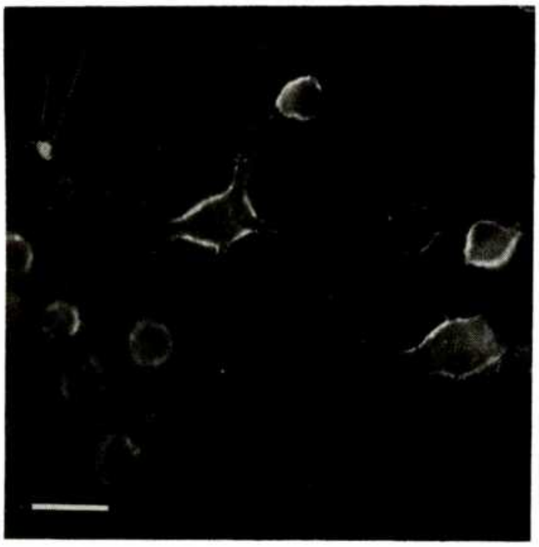

Fig. 3 Immunofluorescence of unfixed mouse spinal cord and dorsal root ganglia cells. After 4 weeks in culture, cells were stained as in Fig. 1 but without fixation. The staining pattern is typical of membrane antigens. Scale bar, 40 μm.

was completely inhibited by a preincubation of the proteins with 10^{-2} M PC, suggesting that PC is part of the determinant. Other MPs of the IgA class (XRPC 24, XRPC 25, UPC 61, EPC 109) did not bind to any cell type in our cultures. MOPC 167 also did not bind the fibroblastic lines, 3T3 4E (ref. 17), cl ID (ref. 18); A9 (ref. 19) and DVGK (a kidney fibroblast-like cell line); a mouse liver cell line, ML311 (established by H. Coon); and a neuroblastoma cell line, N4TGl (ref. 20).

The agreement between our observations and those obtained with accepted markers for neurones indicates that the receptor for PC-BMPs can serve as a marker for neurones in cultured murine nerve cells. It is not yet known whether the antigen is neurone-specific or simply more exposed than on other cell types. An important advantage of our method is that it can be used on live cells (Fig. 3). In addition, with our reagents the labelling was stronger and more specific than labelling with tetanus toxin, which also stained some background cells. It was surprising that although PC is present on the surface of most eukaryotic cells, PC-BMPs reacted only with neurones. Regardless of the reason for this binding, the observation that live cells could also be stained by this method offers the possibility of both identifying and separating neurones from mixed nerve cell populations using methods such as the fluorescence-activated cell sorter[21].

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Morphological and biochemical maturation of neurones cultured in the absence of glial cells

B. Pettmann, J. C. Louis & M. Sensenbrenner

Centre de Neurochimie du CNRS, and Institut de Chimie Biologique,
Faculté de Médecine, 67085 Strasbourg Cedex, France

Cultures of isolated neurones offer possibilities for investigating their properties directly in the absence of glial cells. Furthermore, such a culture provides a suitable model for the study of the biochemical and functional maturation of neuronal cells in a well defined molecular environment. Culture techniques of isolated neurones have been developed for cells originating from the peripheral nervous system[1], especially sympathetic ganglion cells[2-5]. Neurones from the central nervous system (CNS) have proved more difficult to maintain in culture in the absence of other cell types[6]. It is only recently that hippocampal neurones from rat embryos have been successfully maintained in culture[7]. We now describe a method for obtaining a pure culture of dispersed neurones from the telencephalon of 8-day-old chick embryos. This method is derived from a technique previously developed in our laboratory[8]. In those culture conditions, the neuronal cells were obtained by dissociation of cerebral hemispheres from 5–7-day-old chick embryos. The cells remained isolated for only 48 h after plating. Beginning with the 3rd day *in vitro*, the cells clumped together and formed large aggregates in which it was quite difficult to identify the cell types. We have improved those culture conditions and have succeeded in cultivating neurones which remained well dispersed for the whole period of culture.

We established the purity of the neuronal preparation by the morphology and by the binding of tetanus toxin, which is the most specific histological marker for neurones in culture[9]. We studied the maturational pattern of these cultures both morphologically and biochemically.

The two main improvements made to obtain cultures of pure dispersed neurones were the use of older embryos, 8–11 days old, and the technique of preparation of the polylysine substrate. Polylysine-coated surfaces were first developed by Yavin[10] and by Letourneau[11] to improve the attachment of the cells to the tissue culture dish. The polylysine solution was prepared immediately before use by dissolving 10 mg of polylysine (Sigma, type IB or VIIB, PM 70,000) in 1 l of a 0.1 M boric acid–NaOH buffer, *p*H 8.4. After sterilisation by Millipore filtration, 10 ml of the polylysine solution were added to each Petri dish (Falcon, 100 mm diameter) for 24 h at room temperature. After removal of the polylysine solution, the plates were rinsed once with 10 ml of Dulbecco's modified Eagle's medium (DMEM, GIBCO), filled with 6 ml of nutrient medium (80% DMEM + 20% fetal calf serum) and placed in a CO_2 incubator (37 °C, 95% air–5% CO_2). The cell suspension was prepared from the telencephalon of 8–11-day-old chick embryos as previously described[8]. The final concentration of the cell suspension was adjusted to 10^6 cells per ml of nutrient medium. Of this suspension, 4 ml, corresponding to one cerebral hemisphere, were added to each prepared dish and the cultures were immediately placed in the incubator. After 3 days incubation the medium was removed and replaced by fresh medium. The

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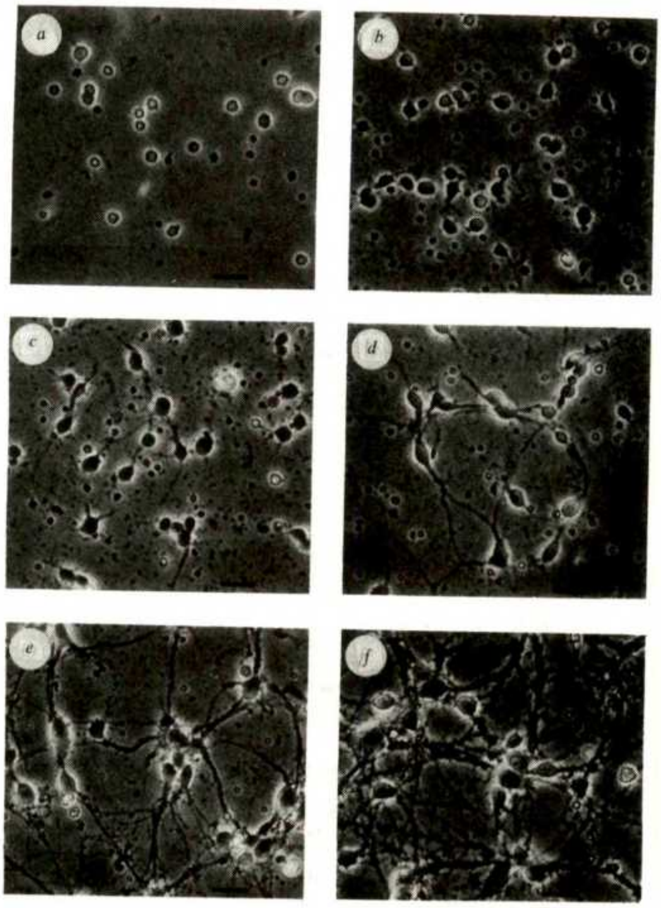

Fig. 1 Morphological development of cultured brain cells dissociated from 8-day-old chick embryos and grown on polylysine-coated Petri dishes. Observations by phase-contrast microscopy. Each bar represents 30 µm. *a*, Initial cell suspension; *b*, 12 h; *c*, 24 h; *d*, 2 days; *e*, 4 days; *f*, 7 days in culture. Detailed culture conditions are described in the text.

cultures remained healthy until the 10th day, as seen by trypan blue coloration.

Figure 1*a* shows that the dissociation of the telencephalon into single cells was complete. The cells adhered very rapidly (within 2–5 min) to the polylysine substrate. After 6 h of incubation, the plating efficiency of the initial suspension was 98 ± 0.5%. The further morphological development of a typical culture is shown in Fig. 1*b*–*f*. The criteria used for the evaluation of the morphological maturation were the size of the cell bodies and the length and complexity of the processes. The outgrowth of processes began between 6 and 12 h (Fig. 1*b*). However, at 12 h, many of the cells showed no indication of neurite formation. Between days 1 and 7, the size of the cell bodies and the number of fibre-bearing cells increased, and the processes spread out and became progressively more ramified, until they formed a dense network covering the entire plate (Fig. 1*c*–*f*).

The most important aspect of our work is the obtention of pure neuronal cultures, as 98% of the cells bind the tetanus toxin (Fig. 2*a*). The absence of glial cells in our cultures could be due partly to the fact that gliogenesis is known to occur later than 8 days during the *in vivo* development of the chick embryo, and partly to the fact that the proliferation in culture of the few glial cells present in the initial cell suspension is inhibited by the polylysine substrate. Non-neuronal proliferation is further prevented by the complete absence of clump formation in our cultures. To estimate the number of glioblasts remaining in the neuronal cultures the cells from a 7-day-old culture were carefully scraped from their dishes, rinsed with serum-free medium, centrifuged at 2,000 r.p.m. for 5 min and transferred to a collagen-coated Petri dish. This substrate is known to favour glial proliferation and maturation. The cells were allowed to grow for 24 h in DMEM containing 20% fetal calf serum and the glial cells present and spread out in the dish were counted under phase-contrast microscopy. The number of glial cells corresponded to less than 0.1% of the total cell population. In any event, our cultures show striking differences from most of those previously described for CNS neurones of embryonic chick or rat (ref. 7 excepted), in which the proliferation of glial cells cannot be entirely inhibited, even in the presence of antimitotic drugs[12,13].

We have correlated the morphological observations with the development of some cellular parameters (Fig. 3). Between the 1st and 3rd day, there is a doubling of the cell number per culture dish and a parallel doubling of the amount of DNA (Fig. 3*a*,*b*). The first change of medium on the 3rd day causes a decrease in the DNA content and cell number. After 4 days these two parameters remain constant. The amount of RNA per plate (Fig. 3*c*) increases until around the 6th day; the amount of protein

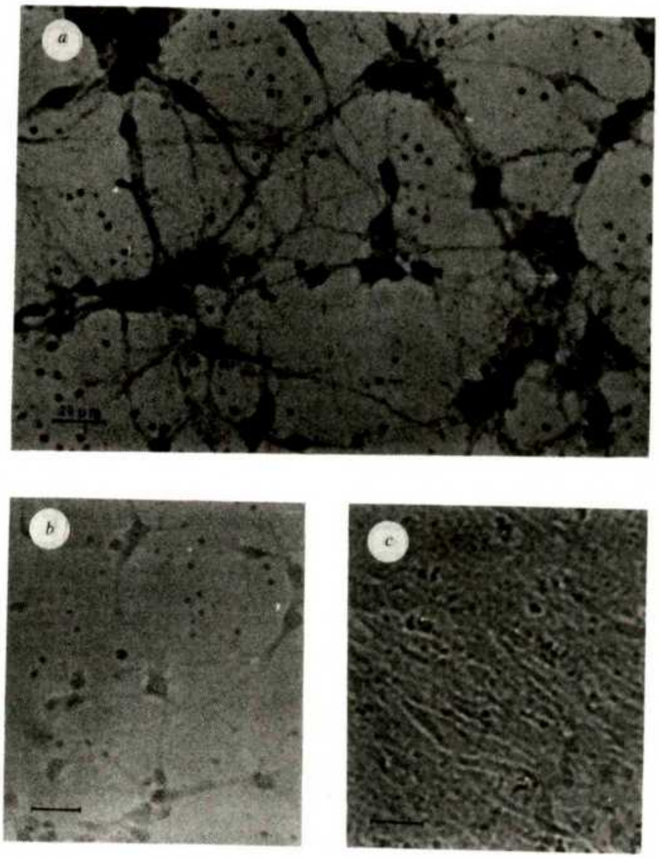

Fig. 2 Immunohistochemical demonstration of tetanus toxin binding sites in the neuronal cultures. *a*, 8-day-old chick embryo cerebral hemisphere neurones cultured for 5 days on polylysine-coated Petri dishes; stained with tetanus toxin, human anti-toxoid gammaglobulins and anti-human gammaglobulins coupled to peroxidase. *b*, 8-day-old chick embryos cerebral hemisphere neurones cultured for 5 days on polylysine-coated Petri dishes; control preparation, stained with tetanus toxin and with anti-human gammaglobulins coupled to peroxidase but without toxoid antiserum. *c*, 20-day-old glial cell cultures, from 15-day-old chick embryo cerebral hemispheres, stained as in *a*. The reaction was always negative in these cultures. Each bar represents 20 µm. The immunohistochemical reaction for the localisation of tetanus toxin binding sites was carried out as described by Mirsky *et al.*[9]. The cultures were rinsed three times with a 0.1 M phosphate buffer, *p*H 7.4, and fixed with a solution of 4% paraformaldehyde and 0.1% glutaraldehyde in 0.1 M phosphate buffer at 4°C for 20 min. After washing, the cells were incubated with 200 µl of tetanus toxin solution (10 $\mu g\ ml^{-1}$ in DMEM), then with either human gammaglobulins or horse antiserum against tetanus toxoid, and finally with either sheep anti-human gammaglobulins coupled to peroxidase (Institut Pasteur) or rabbit anti-horse gammaglobulins conjugated with fluorescein isothiocyanate (Nordic Limited). For each step, the cells were incubated for 30 min at room temperature. Peroxidase activity was revealed by a 0.05% (w/v) solution of diaminobenzidine in 0.1 M phosphate buffer, *p*H 7.4, containing 0.5% (v/v) H_2O_2. In the control preparations, either the tetanus toxin or its antiserum was omitted.

(Fig. 3*d*) increases until the 8th day. These increases occurring after cell proliferation has ceased may be related to the observed increases in the size of the cell bodies and the extension of the processes. Tyrosine hydroxylase activity (Fig. 4) was present in our cultures and showed an eightfold increase between the 1st and 7th days of culture. Dopamine-β-hydroxylase activity was not detected. These morphological and biochemical results indicate the existence of a maturation process, despite the short life span of the cultures.

To our knowledge, as far as the high plating efficiency, the survival of the cells and the purity of the neuronal preparation are concerned, no comparable observations have been reported for central neurones in culture. This culture system was developed with cells originating from the whole telencephalon, but we also obtained pure cultures of neurones from more specific regions of the brain from chick embryos such as striatum, cortex, thalamus, pons–medulla and the optic lobes. Such a culture system will be useful for the study of biochemical and possibly functional maturation of neuronal cells. It will also allow an extensive characterisation of pure neurones, for in the present conditions the neuronal population is not contaminated by glial cells, as is the case with bulk-prepared neurones.

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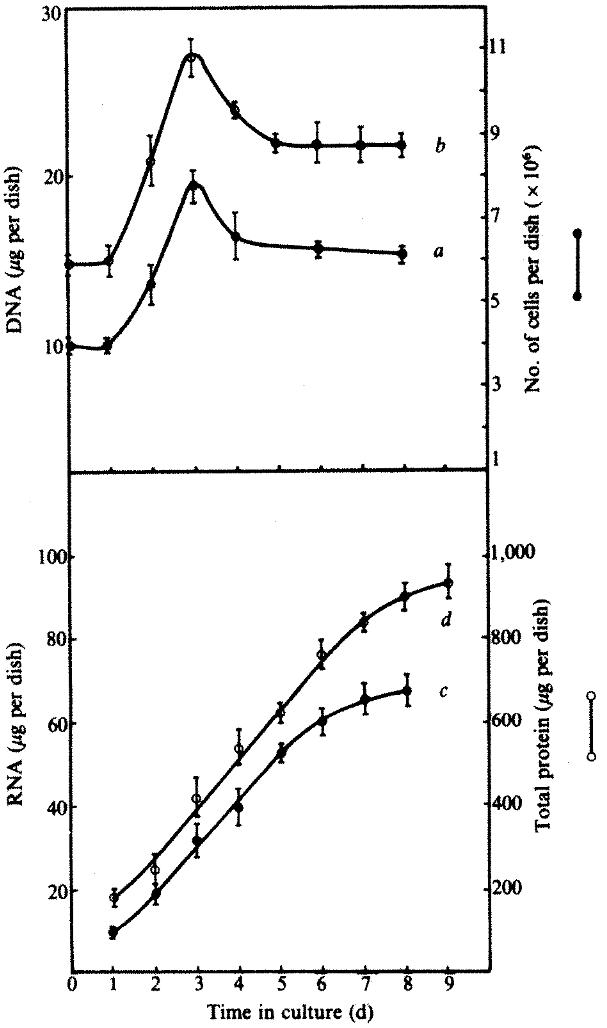

Fig. 3 Biochemical development of isolated neurones in culture. *a*, Number of cells per dish (●). The cells were collected in 10 ml of 0.9% NaCl and aliquots were counted in a Neubauer haemocytometer. Each point represents the mean count ± s.d. of triplicate determinations of three cultures each. *b*, *c*, *d*, Amounts of DNA (○), RNA (●) and total protein (○) per plate, respectively. The cultures were rinsed three times with a 0.9% NaCl solution, collected into 5 ml of 0.9% NaCl and centrifuged at 3,000 r.p.m. for 5 min. The pellet was sonicated twice for ×20 s with a 20-s interval in 1 ml of 2 M NaCl solution containing 5 mM EDTA and 50 mM Tris-HCl (*p*H 8). DNA was determined by the method of Burton[14], RNA by the method of Mejbaum[15] and total proteins by the method of Lowry[16]. Each point represents the mean value ± s.d. of triplicate determinations of six cultures each.

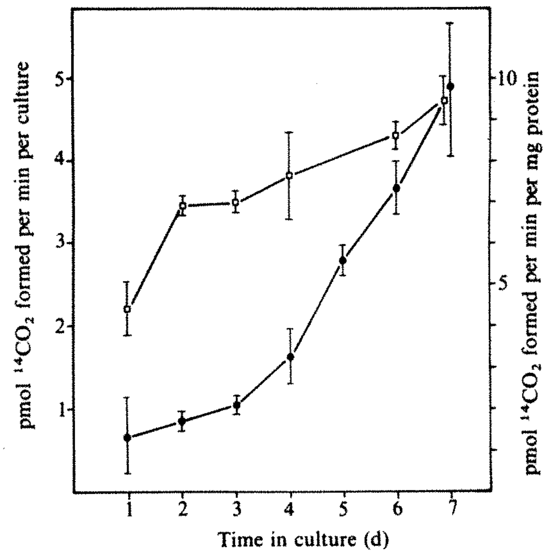

Fig. 4 Developmental pattern of tyrosine hydroxylase (TH) activity in cultures of 8-day-old chick embryo cerebral hemispheres, grown on polylysine, expressed per mg protein (□) and per culture (●). For the determination of TH activity, the cultures were collected in 50 μl of 0.05 M Tris, *p*H 6.0 (acetate), containing 0.2% Triton X-100, homogenised in glass microhomogenisers and centrifuged for 15 min at 12,000*g*. TH activity was assayed on 10 μl (corresponding to 15–40 μg protein) of this supernatant, by the $^{14}CO_2$-trapping method described by Waymire *et al.*[17], with slight modifications. The final concentrations of the incubation mixture were as follows: 0.17 M sodium phosphate buffer (*p*H 6.2), 0.8 mM $FeSO_4$, 0.3 mM pyridoxal phosphate, 30 units ml^{-1} catalase (Boehringer), 0.2 units μl^{-1} dopa decarboxylase (1 unit = 1 nmol $^{14}CO_2$ formed per min) prepared from pig kidney[17], 8.0 mM $DMPH_4$ (Aldrich), 30 mM 2-mercaptoethanol and 150 μM L-[1-^{14}C]-tyrosine (specific activity: 50 mCi $mmol^{-1}$; Amersham). The reaction was carried out for 20 min at 37°C in tubes sealed with rubber injection vial caps. A plastic well was suspended from the rubber injection stopper, which contained 100 μl Protosol (Packard) to trap $^{14}CO_2$ formed during the assay. The reaction was stopped by injecting 100 μl of 3M H_2SO_4 and the tubes were replaced for an additional period of 20 min at 37°C. The Protosol was transferred to counting vials containing 10 ml of Monophase 40 scintillation liquid (Packard). The counting efficiency was 95%. Each point represents the mean value ± s.d. of 8–10 cultures from 3 different experiments. For the determinations of dopamine-β-hydroxylase activity, the cells were homogenised in 5 mM Tris-HCl buffer (*p*H 6.5) containing 0.1% Triton X-100 and the homogenate was assayed according to Bouclier *et al.*[18].

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Properties of haematopoietic stem cells surviving 5-fluorouracil treatment: evidence for a pre-CFU-S cell?

G. S. Hodgson & T. R. Bradley

Biological Research Unit, Cancer Institute, 481 Little Lonsdale Street,
Melbourne 3000, Australia

The spleen colony assay of Till and McCulloch[1], which is a clonal assay[2], is considered to be a measure of the stem cell content of haematopoietic tissue. Stem cells are a heterogeneous population[3–6] with respect to their capacity to generate further stem cells (assayed as colony-forming units in spleen, CFU-S) and progenitors of red cells, granulocytes, macrophages and platelets. It has been suggested that stem cells are organised for use according to their generation age[5,6]. There is evidence to indicate that drugs such as hydroxyurea (HU) and 5-fluouracil (5-FU), which preferentially eliminate proliferating cells, spare non-cycling stem cells which have a high capacity to generate other stem cells[6] and progenitors of granulocytes and macrophages[6] as well as megakaryocytes[7]. We show here, however, that the CFU-S content of haematopoietic tissue is not an adequate predictor of the capacity of that tissue to repopulate bone marrow of a lethally irradiated host. Furthermore it appears that haematopoietic stem cells are not only heterogeneous with respect to their capacity to generate other stem cells and progenitors of haemic cells in the spleen but also differ in their capacity to 'home' to either the bone marrow or spleen. The evidence suggests a class of 'primitive' haematopoietic stem cell (pre-CFU-S) that accumulates in the bone marrow to give rise to cells capable of growing in spleen.

During studies of the properties of the haematopoietic stem cells that survive 5-FU treatment we observed that the number of colonies per spleen, in irradiated mice which had been injected with bone marrow from 5-FU treated donors (FUBM), showed a marked increase between 8 and 14 days after irradiation (Fig. 1*a*). The spleen weights of irradiated mice injected with FUBM also showed a remarkable increase between days 12 and 14 (Fig. 1*b*). In contrast, spleen colony counts remained constant as had been previously noted[8], and spleen weights only increased slightly, in mice injected with normal bone marrow (NBM).

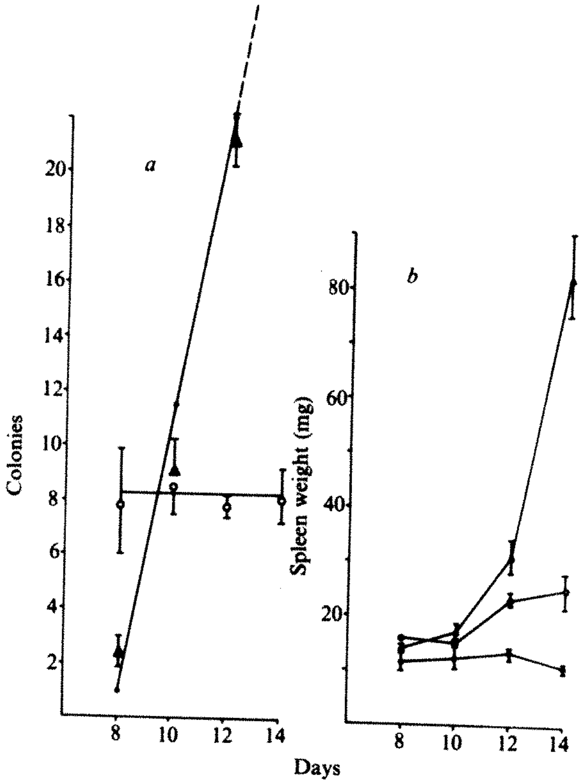

Fig. 1 *a*, Colony counts plotted against time. Bone marrow donors were 10 normal 10–12-week-old (BALB/c × C57BL/6)F_1 male mice or 10 F_1 mice which had been injected intravenously 4 days previously with 5-FU 150 mg per kg. Groups of 10 850R X-ray irradiated F_1 mice, injected with either normal BM, FUBM or uninjected controls were anaesthetised with ether and then killed by cervical dislocation at 8, 10, 12 or 14 days after irradiation, the spleens were removed and fixed in FAA (formalin/acetic acid/alcohol solution) for 24 h then formalin *p*H 7.4. Colonies were counted (using a dissecting microscope ×16.5 magnification). The linear correlation coefficient *r* was 0.8937 for colony counts against time, for FUBM injected mice and 0.1201 for NBM-treated mice. The slope (colonies per day) was 5.33 ± 0.62 for FUBM treated mice and not significantly different from zero for NBM treated mice. *b*, Weights of fixed spleens of mice injected with normal bone marrow or with bone marrow removed 4 days after 5-FU injection. ▲, FUBM injected mice; ○, NBM injected mice; ●, X-ray irradiation controls.

In other experiments using marrow of hydroxyurea-treated donors whose CFU-S have a higher than normal CFU-S generating capacity[6], the phenomenon of time dependence of spleen colony counts was also observed although to a lesser

Table 1 CFU-S[1] and GM-CFC[9] content and marrow (MRA) and spleen (SRA) repopulating ability of femurs and spleen of X-ray irradiated female (BALB/c × C57BL/6)F_1 mice injected with either normal bone marrow (NBM) or marrow obtained from donors injected with 5-FU 1 day previously (FUBM)

		Day 13							
		Spleen			Femur				
Donors	CFU-S dose	Colonies	CFU-S ×10^{-3}	GM-CFC ×10^{-3}	CFU-S ×10^{-3}	GM-CFC ×10^{-3}	MRA ×10^{-3} per femur	SRA per femur
FU–BM 0.2 femur	1.7 ± .4	13.1 ± 1.2	1.2 ± 1.12	137 ± 13	0.72 ± 0.10	52.8 ± 4.2	42.4 ± 7.6	200 ± 40
NBM 0.025 femur	194* ± 11	Confluent	4.03 ± 0.40	177 ± 6	0.190 ± 0.016	7.8 ± 1.3	2.8 ± 0.2	64 ± 13
Radiation control	0	0	ND	0	ND	0†	0†	0†

* The dose of CFU-S in NBM is estimated from a colony count made on day 10 of spleens obtained from irradiated mice injected with 0.0025 femur obtained from normal mice. GM-CFC content was calculated from the mean of five replicate dishes plated with an appropriate aliquot of a pooled femoral marrow cell suspension from 10 mice. † No colonies were seen in five dishes plated with 1/100 of a femur of marrow from irradiation control mice. ND, not determined.

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degree ($r = +0.7961$; slope 3.2 colonies per day) than in the 5-FUBM experiment.

Our results indicate that estimates of CFU-S content of marrow suspensions based on a 10-day spleen colony count are not meaningful where the colony count is varying with time. It is as if the haematopoietic stem cells which survive 5-FU preferentially accumulate in the marrow where they give rise to large numbers of CFU-S, some of which then migrate to the spleen where they give rise to delayed colony development.

In relation to bone marrow-seeking stem cells we have repeatedly observed (see, for example, Table 1) that irradiated mice injected with FUBM (from mice 1 to 4 days after FU treatment), had few (13–22) colonies in the spleen but had a higher content of GM-CFC and CFU-S in their femurs at day 13 than mice injected with suspensions of NBM containing about 200 CFU-S. The interpretation of the assay for CFU-S content of cell suspensions obtained from irradiated mice injected with FUBM was not complicated by a change of spleen colony counts in the secondary irradiated recipients between days 10–14. Marrow from mice injected with a bone marrow suspension taken 1 day after 5-FU injection, which produced only 1.7 spleen colonies at day 10, and 13 at day 13, contained four times more CFU-S and six times more GM-CFC than that of mice injected with a dose of normal bone marrow containing 197 CFU-S (Table 1). The ratio of the CFU-S content of spleen to the colony count at day 13 was 100:1 for mice injected with FUBM and 20:1 in mice injected with NBM (calculated value based on CFU-S dose). The ratio GM-CFC: average spleen colony was 10,000:1 and 1,000:1 for FUBM and NBM respectively.

The marrow (MRA) and spleen (SRA) repopulating ability of marrow cell suspensions prepared from X-ray irradiated recipients injected with either NBM (X+NBM) or FUBM (X+FUBM) was evaluated by injection of cell suspensions into secondary irradiated recipients and measuring the GM-CFC content of femurs and spleen on day 13. The results (Table 1) are expressed as GM-CFC per organ per femur injected. Mice injected with marrow from X+FUBM had ~14 times more GM-CFC in femora and 3 times more in spleen than those injected X+NBM marrow. If one takes 1 femur ≃ 1/20 of total marrow, it can be seen that X+FUBM marrow generates many more GM-CFC in marrow than in spleen, while that from NBM marrow generates as many spleen and bone marrow GM-CFC in secondary X-ray irradiated recipients. Results of primary and secondary transplants show that 'stem' cells, efficient in marrow repopulation are affected minimally, if at all, by a dose of 5-FU that reduces cells capable of forming colonies in spleen at day 10 ('10 day CFU-S'), to about one-thousandth of that of normal.

The non-cycling marrow repopulating stem cells which survive 5-FU treatment are associated with a special class of primitive progenitors, which can form very large colonies (containing 5×10^4 cells) of macrophages in agar after 10 days of culture[10]. These progenitors may prove to be the best '*in vitro*' indicator of the marrow repopulating ability of suspensions of haematopoietic cells.

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Erythropoietin-stimulated erythropoiesis in long-term bone marrow culture

J. F. Eliason, N. G. Testa & T. M. Dexter

Paterson Laboratories, Christie Hospital and Holt Radium Institute, Manchester, UK

The proliferation of multipotential haematopoietic stem cells (CFU-S) is possible in some long-term bone marrow cultures[1–3]. Granulocyte and macrophage progenitors (CFU-C)[2] and megakaryocyte precursor cells (CFU-M)[4] are present in these cultures and undergo full development into mature cells[5–7]. In contrast, while immature erythroid progenitors ('early' BFU-E) are maintained in long-term culture, none of the more differentiated progeny (CFU-E) have been detected, and no morphologically recognisable erythroid cells have been observed[8–11]. We now describe a modified culture system in which the 'early' BFU-E develop into 'late' BFU-E in response to added erythropoietin. Further maturation of these cells into CFU-E and non-nucleated erythrocytes can be achieved by mechanical agitation of the long-term cultures or by transferring the cells into dishes which do not allow cell attachment to occur.

One reason for our previous failure to detect erythroid development in erythropoietin-stimulated long-term marrow cultures[8,9] could be the presence of horse serum in the growth medium used to establish the cultures, which may be inhibitory for erythroid colony growth[11]. On the other hand, fetal calf serum (FCS), which is usually used for the clonal assay of erythroid progenitor cells in semi-solid media, is defective in its ability to stimulate the formation of a competent haematopoietic inductive micro-environment *in vitro* (including endothelial cells, fat accumulating cells and macrophages)[3] and therefore in its ability to sustain *in vitro* haematopoiesis. We have taken advantage of the recent finding that the addition of hydrocortisone to FCS will support maintenance and proliferation of CFU-S and CFU-C in culture (perhaps by influencing the formation of fat accumulating cells in the adherent layer)[12].

The lack of erythropoiesis in long-term marrow cultures may also be related to the finding that the progression from 'early' BFU-E to haemoglobin synthesising cells in erythropoietin-stimulated cultures may require some form of semi-solid support (such as methylcellulose) in the medium[13]. Perhaps the role of methylcellulose in these cultures is to prevent the cells from settling and subsequently attaching to the bottom of the culture dishes. We have used two different approaches to prevent cell adherence in these experiments. The first method was to transfer the cells from the long-term marrow cultures to plastic Petri dishes designed for bacterial culture. Preliminary investigation had indicated that a much smaller proportion of the long-term culture cells will attach to these dishes as compared with standard tissue culture dishes. Our second approach was to place the established long-term cultures on a mechanical shaker to prevent attachment of the erythroid progenitor cells by physical means.

The long-term cultures were established with pooled femoral marrow cells (from 10-week-old male B6D2F$_1$ mice) suspended at a concentration of 1.3×10^6 cells per ml in alpha medium with nucleosides (GIBCO Bio-Cult) supplemented with 25% FCS (Flow), 1% bovine serum albumin (BSA; Path-o-cyte 7, Miles), 10^{-4} M 2-mercaptoethanol (BDH), 7×10^{-6} M human transferrin (Sigma) fully saturated with iron, 10^{-7} M sodium selenite and 10^{-6} M hydrocortisone-21-sodium succinate (Sigma). Aliquots of 10 ml of cell suspension were put into tissue culture flasks (Sterlin), gassed with 5% CO_2 in air and incubated at 33 °C. The cultures were 'fed' at weekly intervals by removing

Table 1 Effect of erythropoietin on erythroid precursor populations in long-term marrow cultures

				Colonies per 10^5 cells				
				CFU-E		BFU-E		
Week	Group	Erythropoietin	Cells per ml $\times 10^5$	day 2	day 4	day 4	day 10	day 14
2	*A*	–	4.2	ND	ND	ND	10 ± 3	22 ± 2
	B	–	4.0	ND	ND	ND	20 ± 3	45 ± 2
	C + D	–	4.0	ND	ND	ND	13 ± 2	28 ± 9
	E	–	3.8	ND	ND	ND	13 ± 3	38 ± 4
3	*A*	–	4.4	0	0	1 ± 1	ND	ND
	B	+	6.4	1 ± 1	2 ± 1	12 ± 1	ND	ND
	C	–	20.2	0.2 ± 0.2	0	0.9 ± 0.4	ND	ND
	D	+	25.0	76 ± 2	ND	9 ± 1	ND	ND
	E	+	7.6	69 ± 4	ND	16 ± 4	ND	ND
4	*A*	–	2.2	0	0	0	7 ± 4	7 ± 1
	B	+	2.0	0	6 ± 2	10 ± 5	5 ± 4	5 ± 4
	C	–	1.7	0	0	0	0	0
	D	+	11.0	41 ± 4	5 ± 3	3 ± 0	0	0
	E	+	2.1	11 ± 2	0	0	0	0

Long-term culture groups: *A*, control; *B*, erythropoietin at week 2; *C*, adherent and suspension cells transferred to Petri dishes at week 2 without erythropoietin; *D*, same as *C* with erythropoietin; *E*, suspension cells transferred to Petri dishes at week 2. For erythroid colony assays, cells were cultured at $1\text{–}2 \times 10^5$ cells ml^{-1} in alpha medium supplemented with 0.9% methylcellulose, 16% FCS, 0.9% bovine serum albumin, 10^{-4} M 2-mercaptoethanol, 7×10^{-6} M transferrin, 10^{-7} M Na_2SeO_3 and 3×10^{-5} M egg lecithin. Four 0.25-ml aliquots of each cell suspension were plated in the wells of Costar (FB-16-24-TC) tissue culture clusters and incubated at 37 °C in a fully humidified atmosphere of 5% CO_2 in air. Colonies derived from day 2 and day 4 CFU-E (1 or 2 clusters of haemoglobin containing cells) and day 4 BFU-E (3 or more clusters) were grown in the presence of 0.5 U ml^{-1} sheep plasma erythropoietin (step III; Connaught). The assay cultures for day 10 and day 14 BFU-E contained 1.8 U ml^{-1} step III erythropoietin and burst feeder activity as supplied by 1×10^6 irradiated cells (15 Gy from a ^{137}Co source)[15]. The results are expressed as the mean ± 1 s.d. ND, not determined.

half the growth medium and replacing it with fresh medium. After 2 weeks of incubation (to allow an adherent layer to develop), 15×10^6 freshly isolated, syngeneic bone marrow cells were added. This time was taken as week zero. At week 2, duplicate cultures were treated according to the following protocols: *A*, control long-term cultures; *B*, erythropoietin-stimulated long-term cultures; *C*, suspension and adherent cells replated in bacterial Petri dishes; *D*, replated suspension and adherent cells with erythropoietin; and *E*, replated suspension cells alone with erythropoietin. The control cultures (*A*) were fed in the usual manner with no further additions. Erythropoietin-stimulated cultures (*B*) were given 10 units of highly purified human urinary erythropoietin which was a hydroxylapatite fraction with a specific activity of 70,000 U per mg of protein. The level of erythropoietin was maintained at 1 U ml^{-1} through week 4 by further addition of erythropoietin with each medium change. Groups *C* and *D* were set up by removing half the suspension cells in growth medium and all of the adherent cells from a third set of long-term cultures (*C + D*) and mixing them with an equal volume of fresh medium. Aliquots of 5 ml were plated in 60-mm diameter bacterial Petri dishes (Sterilin) without (*C*) and with (*D*) 1 U ml^{-1} highly purified erythropoietin. The final group (*E*) contained suspension cells alone from the 2-week-old long-term cultures, diluted with an equal volume of fresh medium, and plated in Petri dishes with 1 U ml^{-1} erythropoietin. The secondary cultures (*C–E*) were re-fed after 1 week by removing half the medium and replacing it with fresh medium. Pooled suspension cells from the long-term cultures were assayed before treatment at week 2, and after treatment at weeks 3 and 4, for erythroid progenitor cells in a modification of the methylcellulose system originally described by Guilbert and Iscove[14,15].

The results of the erythroid colony assays are given in Table 1 and the morphological composition of the cultures at week 3 is shown in Table 2. The 'early' BFU-E (colonies scored at day 10 or day 14) are present in the erythropoietin-treated long-term cultures (*B*) up to week 4 at levels comparable to those found in the control cultures (*A*), as are the numbers of CFU-S (data not shown). The erythropoietin-treated cultures also had significant numbers of intermediate erythroid progenitors, 'late' (day 4) BFU-E and day 4 CFU-E, at weeks 3 and 4, whereas there were essentially none in the control cultures at these times. However, very few day 2 CFU-E or morphologically recognisable erythroid cells were detected in the intact erythropoietin-stimulated long-term cultures. In contrast, the erythropoietin-treated, replated cultures (*D* and *E*) contained many day 2 CFU-E and benzidine-positive erythroid cells including non-nucleated cells were present at week 3.

Since erythropoietin stimulated the formation of haemoglobin-containing cells when long-term culture cells were transferred to bacterial Petri dishes, it was of interest to find whether erythroid maturation could occur in the intact long-term

Table 2 Morphology of suspension cells from erythropoietin-treated long-term cultures at week 3

		Morphology %				
Group*	Erythropoietin	Blasts	Granulocytes	Mononuclear cells	Erythroid cells	**Benzidine-positive non-nucleated cells**
A	–	0	75	25	0	–
B	+	1	96	3	0	–
C	–	1	98	1	0	–
D	+	1	69	2	28	+
E	+	2	82	5	12	+

*As detailed in Table 1.

Table 3 Effect of mechanical agitation on erythropoietin-stimulated erythropoiesis in long-term marrow cultures

Week	Erythopoietin units ml^{-1}	$\times 10^5$ cells per ml	Erythroid colonies per 10^5 cells day 2	Erythroid colonies per 10^5 cells day 4	% Morphology Blasts	% Morphology Granulocytes	% Morphology Mononuclear cells	% Morphology Erythroid cells	Benzidine-positive non-nucleated cells
5	0	8.0	0	0	3	97	0	0	–
	1	12.6	4±3	13±1	0	93	7	0	–
6	0	17.2	0	0	0	95	4	0	–
	0.5	24.0	20±5	15±2	0	64	6	30	+

The cultures were established in Fischer's medium supplemented with 25% FCS and 10^{-6} M hydrocortisone. Four weeks after the second inoculum of bone marrow cells was added, 10 units of sheep plasma erythropoietin (step III) were added to two of the four 10-ml cultures which were transferred to a 37 °C incubator and were placed on a tilting platform shaker (Luckham Ltd). The clonal assays for erythroid progenitor cells were performed as described in Table 1 legend.

cultures if they were mechanically agitated. The results of the shaker experiment are shown in Table 3. After 1 week (week 5) the erythropoietin-treated cultures had high numbers of progenitors capable of giving rise to erythroid colonies at day 4, but no morphologically recognisable erythroid cells were detected in the long-term cultures at this time. On the other hand, by week 6, appreciable numbers of day 2 CFU-E were present in the treated cultures and there were also significant numbers of benzidine positive-erythroid cells. The apparent delayed development of erythroid cells in this experiment, after 2 weeks incubation as compared with only 1 week in the previous experiment (Table 1), is probably due to the impure sheep plasma erythropoietin used in this experiment. This preparation of step III erythropoietin (lot no. 3023-1) may be toxic to the late erythroid progenitors since there are decreased numbers of day 2 erythroid colonies formed by freshly plated marrow cells at erythropoietin concentrations above 0.5 U ml^{-1} in the methylcellulose assay system (data not shown).

Thus we have shown that the addition of erythropoietin to long-term cultures will induce the production of intermediate erythroid progenitors, day 4 BFU-E and CFU-E. However, erythropoiesis seems to be blocked at this stage of maturation because no day 2 CFU-E or morphologically recognisable erythroid cells are found. Transfer of the long-term culture cells to an environment where cell adherence is inhibited allows further maturation of the cells, although there is a rapid decline in the precursor populations as demonstrated by the absence of day 10 and day 14 BFU-E at week 4 (groups *C–E* in Table 1). The latter effect is presumably due to the lack of an effective inductive environment (presumably supplied by the adherent layer in the long-term cultures). The presence of the adherent layer in the long-term cultures is not, of itself, inhibitory to erythroid development, as haemoglobin-containing cells are formed in erythropoietin-treated long-term cultures which have been incubated on a mechanical shaker.

We propose a mechanism for the block in erythroid maturation in the epo-stimulated long-term cultures which involves the erythroid precursors becoming adherent as they mature to the CFU-E stage. The potentially adhering property of late erythroid progenitors is supported by the finding that they are retained on nylon fibre columns[16]. The adherent nature of these cells may be involved in the formation of erythroid islands which can be found in bone marrow and other sites of active erythropoiesis[17,18]. We propose that if, *in vitro*, these cells adhere to unfavourable surfaces (and the adherent layer in the long-term cultures of the tissue culture flask, may be such) further maturation will not occur. On the other hand, if they are prevented from attaching to such surfaces then erythropoiesis can continue.

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Vascular endothelial and smooth muscle cells in culture selectively release adenine nucleotides

J. D. Pearson & J. L. Gordon

ARC Institute of Animal Physiology, Babraham, Cambridge, UK

Endothelial cells in culture can modulate platelet aggregation and vascular tone, in part by producing prostacyclin (PGI_2)[1,2], a powerful vasodilator and inhibitor of platelet aggregation[3,4], but also by their ecto-ADPase activity[5], which initiates the conversion of pro-aggregating ADP to adenosine, a potent vasodilator[6] and platelet inhibitor[7]. We have now demonstrated that cultured aortic endothelial cells exposed to trypsin, thrombin or other stimuli can liberate a high proportion of their adenine nucleotides without substantial loss of lactate dehydrogenase. ADP rapidly accumulates extracellularly, reaching biologically active concentrations before there is further breakdown to adenosine. Whether this selective release of nucleotides is a response to damage, or whether it represents a specific secretory mechanism remains to be resolved. Cultured aortic smooth muscle cells can secrete adenine nucleotides in a similar manner, but extracellular conversion to adenosine occurs much faster.

The production of PGI_2 has previously been demonstrated by the ability of cultured vascular cells to inhibit platelet aggregation induced by a variety of stimuli[1,2,8–10]. Using porcine aortic

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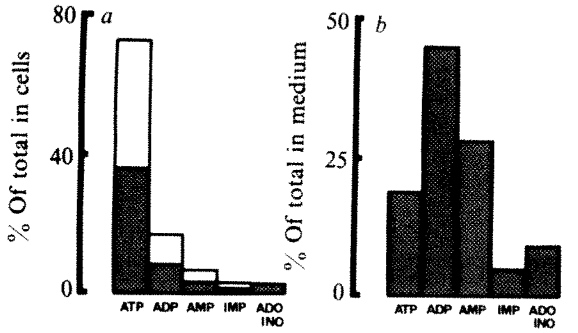

Fig. 1 Adenine nucleotides of cultured porcine aortic endothelial cells were labelled by incubating replicate confluent monolayers in 16-mm diameter wells ($\approx 10^5$ cells per sample) for 60 min with 2 μM ^{3}H-adenosine[11]. The cells were rapidly rinsed three times, then samples were treated with 0.1% trypsin plus 0.025% EDTA in PBS at 37 °C for 5 min, and the supernatant was separated from suspended cells by rapid centrifugation. Purines extracted from the cells before or after treatment with trypsin plus EDTA, or in the supernatant after treatment, were separated by thin layer chromatography and the radioactivity in each fraction was determined[11]. Radioactivity associated with inosine was usually only ~5% of the total, and adenosine and inosine have been combined here (ADO, adenosine; INO, inosine). In *a*, the total height of each column represents the percentage of the total radioactivity incorporated by the cells into each compound. The shaded portion of each column represents the percentage (again of the original radioactivity) in each compound after treatment with trypsin plus EDTA. Thus, the unshaded portion of each column shows the labelled compounds lost from the cells. *b* Shows the distribution of extracellular radioactivity, expressed as percentage of the total release.

endothelial cells in culture[11], we showed that PGI$_2$ production was blocked by low concentrations of aspirin[12], and during further experiments with aspirin-treated cells we found that they could aggregate platelets directly when cell suspensions were prepared from monolayer cultures by a conventional procedure: treatment with 0.1% trypsin plus 0.025% EDTA for 5 min at 37 °C followed by the addition of 0.1 volume of serum to inactivate the trypsin. This aggregation was inhibited by 1–10 μM 2-azido-AMP, which is an antagonist of the ADP receptor on platelets[13], and when cell-free supernatants were prepared by centrifuging, these contained a stable pro-aggregatory compound whose action was similarly blocked by 2-azido-AMP. The aggregation response was almost abolished when the supernatant was incubated for 50 min with 100 μg ml^{-1} apyrase (Sigma, grade I). Treatment with 10 μg ml^{-1} apyrase resulted in the response being initially potentiated (>200% of the control after 15 min) then reduced after longer incubation. This suggested that ATP and ADP were released from the endothelial cells by the procedure used to detach them; the ATPase activity of the apyrase was three to five times its ADPase activity (according to Sigma), and the results were therefore consistent with an initial conversion of ATP by apyrase and slower degradation of the ADP thus formed, together with that originally present.

To investigate the release of nucleotides in more detail, we took advantage of the fact that endothelial cells have a high-affinity transport process for adenosine through which ^{3}H-adenosine is predominantly incorporated into ATP and ADP within the cells[11]. Cell suspensions were prepared from prelabelled monolayers by treatment with trypsin and EDTA as above; the cells were then separated from the extracellular medium by centrifuging, and the cellular and extracellular amounts and distribution of ^{3}H measured by thin layer chromatography and liquid scintillation counting[11]. The fraction of the total cellular radioactivity released varied in different experiments (usually 10–30%, occasionally >50%) but the pattern of ^{3}H release was highly consistent. In all experiments the main component lost from the cells was ^{3}H-ATP (Fig. 1*a*), but the largest fraction appearing extracellularly was ^{3}H-ADP (Fig. 1*b*). Release of up to 60% of the intracellular ^{3}H, in a similar pattern, also occurred when cells were suspended by physical disturbance with a rubber policeman. To check that the radioactivity released did not represent a small pool of preferentially labelled nucleotides, we measured the total ATP content of cells and extracellular medium using luciferase[14]. The total cellular ATP concentration was ~4 nmol per 10^6 cells, of which up to 50% was released when cell suspensions were prepared using trypsin plus EDTA as described above.

The high fraction of cellular nucleotides released when the cells were suspended with trypsin plus EDTA was not accompanied by any evidence of cell damage as measured by vital dye exclusion (no detectable staining of cells with trypan blue) or cytoplasmic enzyme leakage (extracellular lactate dehydrogenase activity was <5% of the total cellular activity). Thus, the cells could liberate up to half their adenine nucleotides without the plasma membrane becoming significantly permeable to compounds of higher molecular weight. This selective release of nucleotides was not simply a consequence of the cells becoming detached from their substratum: there was always a small but significant release of nucleotides from prelabelled cells on replacing the medium above the monolayer with fresh medium, and this release was increased by adding agents such as collagenase or thrombin that did not detach the cell monolayer. For example, the addition of fresh culture medium alone at 37 °C for 5 min induced 7.2 ± 1.4% release of incorporated ^{3}H; medium containing 0.2% collagenase released 14.0 ± 0.7% (mean ± s.d., $n = 4$). Treatment with 1–5 U ml^{-1} thrombin for 5 min at 37 °C released up to 25% of ^{3}H without visibly affecting cell morphology. The pattern of extracellular nucleotides found in these experiments was very similar to that obtained using trypsin plus EDTA. Nucleotide release was temperature dependent; for example, the addition of phosphate-buffered saline (PBS) alone for 5 min at 37 °C released 5.3 ± 0.7% whereas at 4 °C release was 2.3 ± 0.7% (mean ± s.d., $n = 4$). Release was not reduced when cells were treated with trypsin and EDTA in PBS containing 10 mM ATP (test release 26.5 ± 3.0%; release in the absence of ATP 24.3 ± 4.8% (mean ± s.d., $n = 4$)). Also, there was no uptake of labelled exogenous nucleotide in conditions inducing selective release of intracellular nucleotides: when PBS containing 1 μM–4 mM ^{3}H-ATP (~2×10^6 d.p.m.) plus 10–100 μM dipyridamole was added to an endothelial monolayer for 5 min at 37 °C, the presence of trypsin plus EDTA did not result in any significant increase in the radioactivity associated with the cells (for example, 0.15% in control, 0.14% with trypsin plus EDTA, means of three observations). Dipyridamole, a potent inhibitor of adenosine uptake by endothelial cells[11], was added to prevent uptake of any ^{3}H-adenosine formed during the incubation with ^{3}H-ATP,

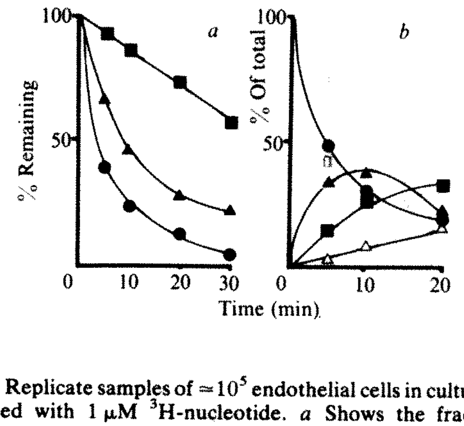

Fig. 2 Replicate samples of $\approx 10^5$ endothelial cells in culture were incubated with 1 μM ^{3}H-nucleotide. *a* Shows the fraction of substrate in serum-free HEPES-buffered Dulbecco's medium[23] remaining unchanged outside the cells at various times after incubation. *b* Shows the pattern of metabolites outside cells incubated with ATP in PBS containing 1.8 mM Ca^{2+}. ●, ATP; ▲, ADP; ■, AMP; △, adenosine. Dipyridamole (10 μM) was present in all experiments.

because we had confirmed (see below) the recent report[15] that endothelial cells could metabolise extracellular ATP to adenosine.

It was clear that nucleotide metabolism occurred either during or after release, because the pattern of intracellular nucleotides secreted was (as shown in Fig. 1) different from that found outside the cells. This difference was even more striking when similar experiments were carried out using cultured pig aortic smooth muscle cells: up to 50% of the ^{3}H secreted by smooth muscle cells was detectable after 5 min as ^{3}H-adenosine, although again the main intracellular change was a loss of ^{3}H-ATP. We did, however, establish that at shorter time intervals most of the label released was ATP. To investigate the extent of extracellular metabolism of adenine nucleotides in more detail, cultures of endothelial cells or smooth muscle cells were incubated with individual labelled nucleotides and the metabolites in the culture medium determined.

Figure 2*a* shows that endothelial cells catabolised added ATP, ADP or AMP and the half lives were, respectively, <5, ≈ 10 and >30 min at 1 μM substrate concentration. With smooth muscle cells the half life for each nucleotide was ≈ 5 min in the same conditions. Metabolism of added ^{3}H-ATP by endothelial cells resulted in an initial transient peak of ^{3}H-ADP, followed by a more prolonged peak of ^{3}H-AMP and finally a progressive increase in ^{3}H-adenosine (Fig. 2*b*). When the same experiment was carried out using smooth muscle cells, the peaks of ^{3}H-ADP and ^{3}H-AMP were much smaller and never exceeded the amount of adenosine formed.

Taken together, these results suggest sequential degradation of extracellular nucleotides (ATP → ADP → AMP → adenosine) by both endothelial cells and smooth muscle cells, although the relative rates of breakdown differ significantly in the two cell types. The pattern of extracellular nucleotides found after 5 min trypsinisation of each cell type is consistent with the transient release of ATP, and a smaller amount of ADP, followed by extracellular metabolism.

Our findings raise an important caveat for experiments with platelets and cultured cells. We, and others, have previously used a platelet aggregation bioassay to estimate the production of PGI_2 by cultured vascular cells, in which the cells are usually suspended by conventional procedures similar to those described in the present study—the use of trypsin, collagenase, EDTA or a rubber policeman[1,2,8–10]. As we have now demonstrated a hitherto unsuspected selective release of adenine nucleotides from cultured vascular cells, affecting platelet aggregation, this must be considered in future experiments using this method.

The selective release of ATP from cultured endothelium seems to be a cellular response to potentially damaging stimuli. It is not a nonspecific increase in membrane permeability like that associated with cell lysis, because the cells remain viable and there is very little release of cytoplasmic protein. Nor is it apparently a secretory process involving degranulation—we found no evidence for compartmentalisation of endothelial ATP. We also failed to demonstrate any inward transport of ATP across the endothelial plasma membrane; this implies that the selective release may represent a unidirectional increase in membrane permeability to nucleotides. The release process could be in some respects analogous to the selective release of prostaglandins from stimulated cells, but the precise mechanism involved remains to be determined.

Whatever the mechanism responsible, the release of nucleotides from vascular endothelium *in vivo* would have great biological significance, because adenine nucleotides and adenosine not only influence platelet aggregation but are also potent vasoactive agents. Efflux of adenosine, with smaller quantities of nucleotides, has been measured in several perfused vascular preparations[16–18], particularly in the vasculature of cardiac or skeletal muscle during hypoxia, where the circulating adenosine is thought to act as a local hormone to increase blood flow[19–21]. Although adenosine itself might be released from endothelium or muscle cells, perhaps by the uptake process operating in reverse, this seems unlikely, as the intracellular concentration of adenosine is very low ($<1\%$ of the ATP concentration), and the reactive hyperaemia caused by endogenous adenosine during hypoxia is potentiated by inhibitors of adenosine transport such as dipyridamole[22]. An alternative pathway would be the selective release of a small proportion of the cells' ATP with subsequent conversion to adenosine by the ectoenzymatic cascade. Further work is needed to test whether this pathway, which we have observed *in vitro*, operates *in vivo* (for example, in response to hypoxia) to regulate vascular tone.

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Orthotopic bone induction at sites of Moloney murine sarcoma virus inoculation in mice

K. Wlodarski

Department of Histology and Embryology,

M. Kobus & M. Luczak

Department of Virology, Institute of Biostructure, Medical School, Warsaw, Poland

Moloney murine sarcoma virus (M-MSV) induces at the site of inoculation in newborn[1,2] and adult[3–5] mice and rats various types of sarcoma[1,6], including osteosarcoma[7,8]. The induced tumours are fatal in newborn mice[3,9], whereas sarcomas developed in adult animals regress spontaneously[3,5,10–12]. The regression is mediated mainly by a cellular response[3,5,10–12]. We have now demonstrated that the presence of sarcomas induced by M-MSV is a powerful stimulus for periosteal osteogenesis around tumour masses. Orthotopically induced osteogenesis by M-MSV may serve as a model for local regulation of bone growth and for cell differentiation studies, and may help explain the aetiology of some human bone disorders.

Inbred 5-week-old NMRI mice of both sexes were inoculated into left shanks with 0.2 ml of standard M-MSV diluted 1 : 5 in phosphate-buffered saline. The virus was prepared according to Chanaille *et al.*[13] (10^3 TID_{50} in 0.2 ml). Mice were killed at various intervals between the 3rd and 190th days after inoculation and both shanks, one inoculated with M-MSV and the intact contralateral control, were fixed in Susa solution. Paraffin

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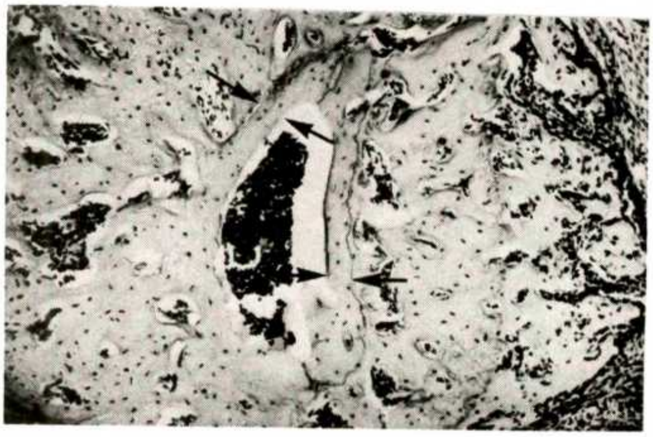

Fig. 1 Periosteal cancellous bone formation in ulna, 16 days after M-MSV inoculation. Arrows indicate the original width of the ulna cortex. ×60.

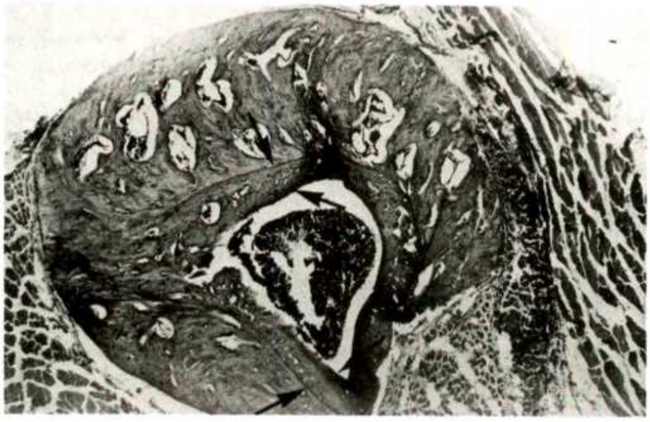

Fig. 2 Tibia, 2 months after M-MSV inoculation and 1 month after tumour regression. Arrows indicate the original width of the tibia cortex. ×38.

sections from the middle of the shanks were cut perpendicularly to the tibial axis, and stained with haematoxylin and eosin. As an additional control five mice were inoculated with heat-inactivated M-MSV (heated at 56 °C for 2 h).

Of 36 mice inoculated with active M-MSV, 34 developed tumours (95%). Tumours grew progressively up to days 14–16, then declined and disappeared completely by days 25–32. Tumours were pleomorphic and had large elongated and round cells with a single hypertrophic nucleus and distinct nucleoli, small fibrosarcoma-like cells predominated. Neoplastic cells were infiltrated with numerous neutrophils. A massive destruction of muscles was always observed during the progressive phase of tumour growth. The regression of tumours was associated with regeneration of muscles, the disappearance of neoplastic cells, and the accumulation of macrophages, fibroblasts and lymphocytes. No heterotopic osteogenesis was observed either during progressive tumour growth or during regression.

A virus-induced sarcoma acts as a very powerful stimulus for orthotopic periosteal osteogenesis. In all cases the presence of tumours was associated with new bone formation in the periosteum of the tibia and/or ulna. In control bones the periosteum was composed of two to four layers of fibroblasts. At 6 days after M-MSV inoculation the periosteum became a thick membrane, composed of 7–20 layers of osteoblasts and fibroblasts; mitotic forms were frequent and osteoid deposition had begun. Bone formation continued until day 16. By this time the newly formed cancellous bone covering the old original cortex was three to five times thicker than the original bone (Fig. 1). The orthotopic bone formation was uneven, sometimes affecting the whole periphery, but always taking place on sites which faced the tumorous tissue. Newly formed orthotopic bone ossicles were also sites of myelopoiesis. Bone formation ceased with tumour regression. During tumour regression the remodelling processes converted induced trabecular bone into more mature forms. Orthotopically induced bone also stopped growing and became covered with periosteal membrane of normal histology (Fig. 2).

Tumours did not form in shanks inoculated with heat-inactivated M-MSV, and there was no periosteal stimulation or bone formation. In the two mice in which inoculation of active M-MSV did not produce tumours, no changes in bone and periosteum histology were observed.

Orthotopically induced bone persisted throughout the 6-month observation period, remained unresorbed, and did not exhibit neoplastic changes. The results obtained may explain some aspects of the aetiology of osseous deformation, such as exostoses. It is not clear whether proliferation of the periosteum is caused by the virus itself, or whether it is mediated by tumour and/or inflammatory cells. M-MSV does not induce osteosarcomas when administered intramuscularly, but when administered into the medullar cavity of newborn rat tibia the virus induces highly malignant osteosarcomas[8]. The development of tumours precedes the stimulation of the periosteum, however, which suggests a direct mechanism of periosteal stimulation.

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Growth of a human mammary tumour cell line in a serum-free medium

David Barnes & Gordon Sato

Department of Biology, Q-058, University of California, San Diego,
La Jolla, California 92093

The MCF-7 line of human mammary carcinoma cells is a well characterised line[1] which has retained a number of the properties expressed by breast epithelium *in vivo*, making it a good model for the *in vitro* study of normal and neoplastic mammary cells. MCF-7 cells have been reported to possess receptors for oestrogen, androgen, progesterone, glucocorticoid[2], insulin[3] and L-3, 3′, 5-triiodothyronine (T$_3$)[4]. These cells also show responses to prolactin[5], and dome formation[1]. This line, like most cells in culture, requires the addition of a serum supplement to the medium for growth. The complex and undefined nature of serum adds a complicating aspect to the design and interpretation of any experiments aimed at understanding the interactions of hormones or drugs with mammary epithelium. Our laboratory has shown that it is possible to replace the serum requirement for a number of cell lines with mixtures of hormones and purified serum factors[6–8]. We now report that MCF-7 cells may be grown, without a lag or adaptation phase, in a serum-free medium supplemented with physiological levels of insulin, transferrin, epidermal growth factor (EGF), prostaglandin F$_{2\alpha}$ (PGF$_{2\alpha}$) and cold-insoluble globulin (CIg). This medium will support a growth rate identical to that of cells in medium supplemented with an optimal concentration of fetal calf serum. The further addition of the α-1 serum protein first purified by Holmes[9] results in a morphology identical to that of cells in 10% serum. Turkington has previously shown EGF effects on mouse mammary explant cultures[10], and mouse EGF has been reported to be stimulatory for human mammary tumour cells[11]. Insulin and transferrin have been shown to be required for the continuous serum-free growth of the ZR-75-1 human mammary tumour cell line[12]. We believe that this is the first report of the effects of transferrin, PGF$_{2\alpha}$, CIg, EGF or the α-1 protein on the MCF-7 line.

Figure 1 shows the growth over an 8-d period of MCF-7 cells in medium supplemented with 10% fetal calf serum or with the five factors. The doubling time in both conditions was about 36 h. This generation time agrees with that reported by other laboratories for these cells[13]. Addition of the five factors to cells in medium with 10% fetal calf serum did not increase cell number beyond that seen with 10% serum alone (data not shown). In serum-free medium in the absence of the five factors cell death occurred after 4 d. Cell growth in the serum-free medium began to slow 6 d after plating and fell behind that of serum by day 8. In most experiments cell number in serum-supplemented medium at any point in the growth curve was higher than that of cells in the serum-free medium. This reflected a higher initial plating efficiency in serum-supplemented medium compared with serum-free medium, but did not represent a significantly lower doubling time in the serum-free medium. The synthetic nutrient medium used in these experiments was a 1:1 mixture of Ham's F12 and Dulbecco-modified Eagle's medium (DMEM) supplemented with antibiotics, 1.2 g l^{-1} sodium bicarbonate, 15 mM HEPES, *p*H 7.4, and 10^{-8} M sodium selenite. No growth was seen in DMEM supplemented with the five factors, and growth in F12 supplemented with the five factors was 80% of that seen in the mixture of the two.

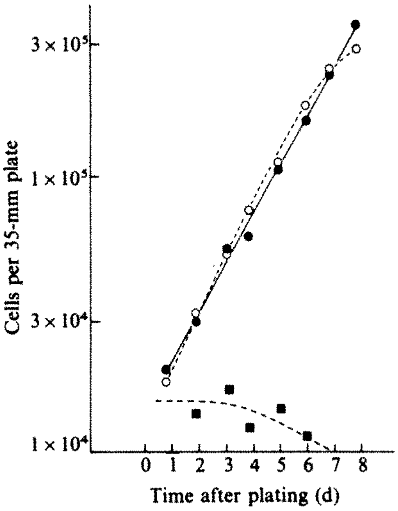

Fig. 1 Growth of MCF-7 cells in serum-free medium. Stock cultures for experiments were grown in a one to one mixture of F12 and DMEM supplemented with antibiotics, 15 mM HEPES *p*H 7.4, 1.2 g l^{-1} sodium bicarbonate and 10% fetal calf serum (Reheis). Cells were detached from stock flasks with a trypsin-EDTA solution in phosphate-buffered saline, treated with trypsin inhibitor, and plated at 3×10^4 cells per 35-mm diameter plate in 2 ml medium supplemented with 250 ng ml^{-1} insulin (Sigma), 25 μg ml^{-1} transferrin (Sigma), 100 ng ml^{-1} EGF (Collaborative Research), 100 ng ml^{-1} PGF$_{2\alpha}$ (Sigma) and 7.5 μg ml^{-1} CIg (Collaborative Research) (○). Control plates contained medium with 10% fetal calf serum (●), or medium with no stimulatory supplements (■). Data shown are the average of triplicate samples. Deviation of single samples from the average was about 5%. For counting, cells were treated with trypsin-EDTA, sheared through a 22-gauge hypodermic needle to produce a single cell suspension, and counted in a Coulter counter. Cell suspensions treated in this way gave counts identical to those counted with a haemocytometer.

Table 1 Effect of insulin, transferrin, PGF$_{2\alpha}$, EGF and CIg on growth of MCF-7 cells in serum-free medium

Conditions	Cells per 35-mm plate ($\times 10^{-4}$)
No additions	4.4 ± 0.1
Insulin, transferrin, EGF, PGF$_{2\alpha}$, CIg	35.0 ± 0.9
Insulin omitted	11.4 ± 1.4
Transferrin omitted	28.4 ± 2.3
PGF$_{2\alpha}$ omitted	30.4 ± 2.0
CIg omitted	27.7 ± 1.0
EGF omitted	34.6 ± 2.4
10% fetal calf serum	40.0 ± 2.8

Cells were plated at 3×10^4 per 35-mm diameter plate in medium with the indicated additions. Concentrations of the factors were the same as that of Fig. 1. Cells were counted 7 d after plating. After 14 d (medium changed on day 8), cell number in medium with the five factors was 85.1×10^4 per plate. In medium with EGF omitted cell number was 78.8×10^4 per plate.

The individual omission of either insulin, transferrin, PGF$_{2\alpha}$, or CIg from the medium resulted in decreased cell number after 7 d (Table 1). Longer incubations (10–14 d) were required to demonstrate EGF effects. EGF effects could not be observed at earlier times if insulin was omitted or the insulin concentration lowered. Insulin was clearly the most important component of the defined medium. In dose–response studies carried out in the presence of the other factors, the maximal effect of insulin was observed with 100–300 ng ml^{-1} with an easily measurable effect at 3 ng ml^{-1}. Transferrin produced a maximal stimulation at 25 μg ml^{-1} with a measurable effect at 1 μg ml^{-1}. The CIg effect was maximal at 7.5 μg ml^{-1} with a measurable effect at 0.75 μg ml^{-1}. CIg at these concentrations is also stimulatory for the RF-1 rat ovary cell line[14] and a mouse embryonal carcinoma line[15]. The effect of CIg on cell number in Table 1 was not due to decreased shedding of cells into the medium. The number of cells floating in the medium was less than 2% of the total number in the dish and this percentage was unchanged by omission of CIg from the medium. The transferrin concentration used in the

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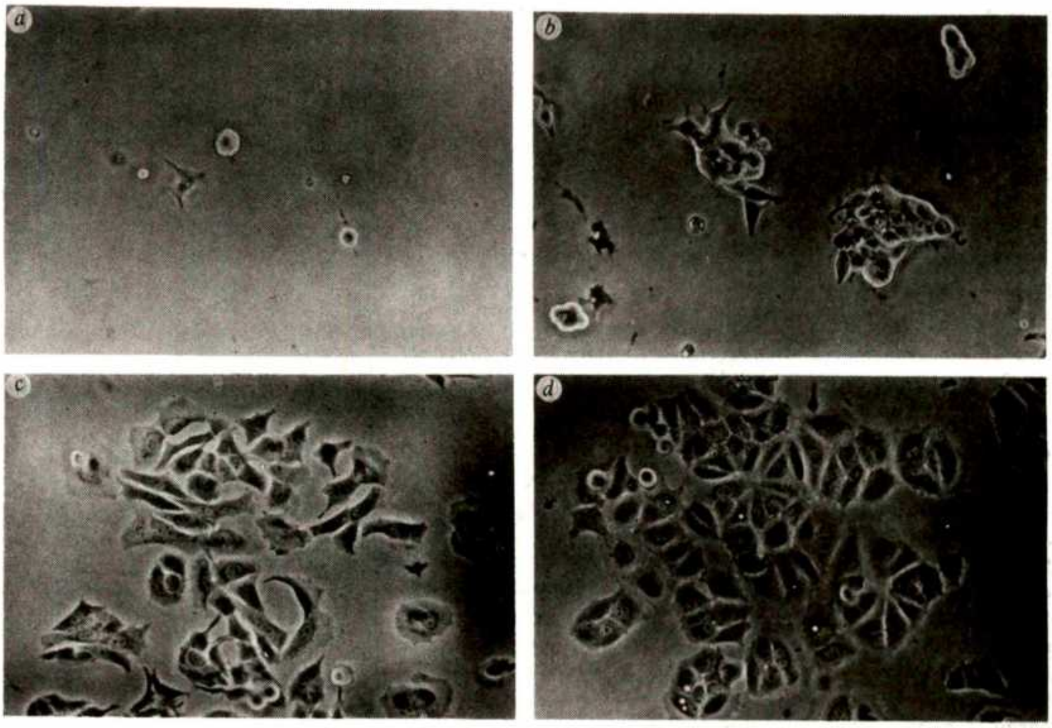

Fig. 2 Effect of the α-1 protein of Holmes on morphology of MCF-7 cells in serum-free medium. Cells were incubated for 3 d in (*a*) medium with no stimulatory supplements, (*b*) medium with the five factors as described in Fig. 1, (*c*) medium with five factors plus the α-1 protein (GIBCO), and (*d*) medium with 10% fetal calf serum. The human α-1 protein was used at a dilution of 1:10. Protein concentration of the α-1 protein in the medium, calculated from information provided by the vendor, was about 250 ng ml^{-1}.

serum-free medium (25 μg ml^{-1}) is about 1/10th (ref. 16), and the CIg concentration (7.5 μg/ml^{-1}) about one-third[17], that which would be found in medium containing 10% serum. PGF$_{2\alpha}$ was maximally stimulatory at 100 ng ml^{-1} and EGF at 10–100 ng ml^{-1}. Both of these factors showed some stimulation at 1 ng ml^{-1}. No increase in cell number was seen on addition of physiological levels of other hormones which have effects on mammary epithelia, including human prolactin, porcine relaxin and vasopressin.

Cells in serum-free medium with the five factors grew as rounded clumps which were poorly attached to the dish, or groups of cells which were attached but poorly spread. The addition of the α-1 serum protein, which has been reported to enhance the growth and spreading of HeLa cells[9], resulted in a striking change in morphology (Fig. 2). This protein greatly improved attachment and spreading of MCF-7 in serum-free medium at concentrations of 1 μg ml^{-1} or less.

Increased cell number has been reported for MCF-7 under various circumstances upon addition of insulin[18], oestrogen[19], androgen[20] or T$_3$ (ref. 4). The androgen effect is reported to be mediated by the oestrogen receptor[21]. Morphological effects of both T$_3$ and oestradiol at concentrations as low as 10^{-10} M were observed in our medium containing the five factors, but no increase in cell number was seen after incubations as long as 14 d, with a medium change on day 7. However, additions of oestradiol at 10^{-9} M did result in an increase in cell number if the oestrogen was added to cells plated in the α-1 serum protein alone (W. Desmond, D. B. and G. S. in preparation). Although it is possible that long-term cultivation of MCF-7 cells in our serum-free medium may expose additional requirements for other hormones, these cells have so far been successfully carried for over 3 months (15 passages) in the serum-free medium.

If great care is taken on transfer to insure removal of residual trypsin, proper control of medium *p*H and temperature and to minimise the amount of time they are out of the incubator, the cells may be continually passaged in the serum-free medium without loss of viability or lengthening of generation time. When cells were grown for five passages in the serum-free medium and then plated in medium with 10% fetal calf serum or medium with the five factors, cell number after 6 d in plates with medium with the five factors was 80% of the cell number in plates with 10% serum.

Allegra and Lippman[12] have described a serum-free medium for ZR-75-1 human mammary tumour cells which includes insulin, transferrin, dexamethasone, T$_3$ and oestradiol. This medium will not support the growth of MCF-7. Although it is not known if the ZR-75-1 line will grow in the medium devised for MCF-7, preliminary experiments indicate that, in addition to MCF-7, another human mammary carcinoma cell line, BT20, also grew in serum-free medium supplemented with insulin, transferrin, EGF, PGF$_2\alpha$, CIg, and the α-1 protein.

A serum-free medium for MCF-7 cells should be useful for several reasons. This medium will greatly facilitate pharmacological and nutritional studies of MCF-7. Studies of hormone binding and subsequent biological effects may now be carried out on healthy, growing, non-selected cells in the absence of interfering hormones or serum proteins. Serum-free medium will allow the easy identification and isolation of products synthesised and secreted by the cells into the medium. Finally, a serum-free medium for human mammary tumour cells may allow the isolation of new lines of normal or neoplastic human mammary epithelium in the absence of other contaminating cell types.

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Platelet-derived growth factor prevents G_0 growth arrest

Charles D. Scher & Marilyn E. Stone

Department of Hematology-Oncology, Sidney Farber Cancer Institute and Children's Hospital Medical Center, Department of Pediatrics

Charles D. Stiles

Laboratory of Tumor Biology, Sidney Farber Cancer Institute, Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts 02115

Baserga[1] has summarised evidence that there are two growth states in which cells have a diploid content of DNA; G_1—the interval between mitosis and S phase in exponentially growing cultures—and G_0, a quiescent state entered only when conditions for growth are suboptimal. G_0 can be distinguished from G_1 by temporal measurements; for BALB/c 3T3 cells, the lag time between G_0 and S phase is never shorter than 12 h (ref. 2), whereas in exponentially growing cells the mean lag time between mitosis and S (G_1 by definition) lasts 5–6 h (ref. 3). G_0 can also be distinguished from G_1 by some biochemical parameters including G_0- and G_1-specific intracellular proteins[4,5]. SV40- or polyoma virus-transformed cells cannot enter G_0 to become quiescent[6–9]. Serum induces the growth of BALB/c 3T3 cells in tissue culture. It sustains the growth of exponentially replicating populations, and causes density-inhibited cells to leave G_0 and replicate[10–12]. Serum contains several sets of hormonal growth factors which have recently been defined[2,13,14]. One of these hormones, the platelet-derived growth factor (PDGF), is released into serum during the clotting process; PDGF is absent in platelet-poor plasma, the liquid portion of unclotted blood[15–19]. It promotes the growth of G_0-arrested cells[2,16] by stimulating cells to become 'competent' to enter the S phase[2]; plasma allows these competent cells to progress through G_0/G_1, synthesise DNA[2,20] and divide[13]. We now show that PDGF has a second function. It prevents replicating cells from entering G_0.

Density-inhibited cultures of BALB/c 3T3 cells (clone A31) were treated for 5 h with platelet extract containing partially purified PDGF to induce the majority of the cells to become competent for replication. Transfer of these cells to medium containing an optimal concentration (5%) of dialysed platelet-poor plasma allows these cells to begin to enter the S phase after a lag of 12 h (refs 2, 20, 21). Addition of the dihydrofolate reductase inhibitor methotrexate[22] (100 nM) prevented DNA synthesis, and flow microfluorimetry demonstrated that the growth-arrested cells had an apparent G_1 content of DNA (data not shown). The methotrexate was removed and plasma-supplemented medium containing hypoxanthine and thymidine was added; duplicate cultures were treated with ^{3}H-thymidine (TdR) or colchicine for 1 h, at various times after removal of the methotrexate, and fixed[2]. DNA synthesis, as determined by autoradiography, was detected in 87% of the cells within the first hour after removal of the methotrexate (Fig. 1). The percentage of cells synthesising DNA remained constant for 6 h and then fell abruptly. By 10 h, little DNA synthesis was detected, indicating that the S phase had been completed. Mitosis was somewhat less synchronous, beginning at 9 h, reaching a peak at 11 and ending by 15 h. Treatment of cells with PDGF after the removal of methotrexate did not alter the kinetics of DNA synthesis or mitosis (data not shown).

Cells arrested by plasma withdrawal shortly before the onset of DNA synthesis enter the S phase with first-order kinetics, whereas cells blocked in early S phase begin DNA synthesis in a synchronous fashion[20,23]. Thus, the data indicate that methotrexate blocks cells near the G_1/S phase boundary in early S phase.

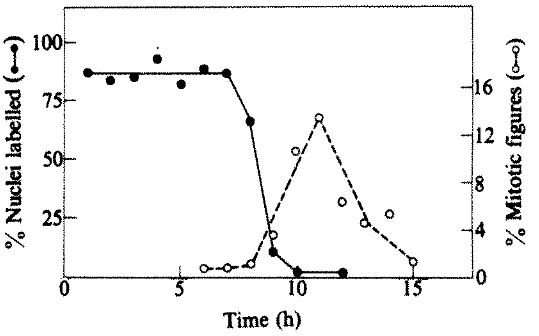

Fig. 1 Methotrexate causes growth arrest in early S phase. BALB/c 3T3 cells (clone A31) were grown to confluence on Falcon microtitre wells (area 0.3 cm^2) in Dulbecco's modified Eagle's medium containing 10% calf serum[2]. Density-inhibited cultures were treated with 150 μg ml^{-1} of heat (100 °C)-treated platelet extract containing PDGF for 5 h, washed once and transferred to medium containing 5% (v/v) dialysed human plasma containing 100 nM methotrexate (National Cancer Institute). After 18 h all cultures were transferred to fresh medium containing 5% plasma, 106 nM hypoxanthine and 16 nM thymidine. ^{3}H-TdR (5 μCi ml^{-1}) was added to duplicate cultures at hourly intervals after removal of the methotrexate. The cells were fixed 1 h after addition of ^{3}H-TdR at the time shown, washed twice with 5% trichloroacetic acid and processed for autoradiography. Duplicate cultures were treated with 2 μM colchicine (GIBCO) for 1 h and fixed at the time shown; the percentage of mitotic figures was determined.

Density-inhibited BALB/c 3T3 cells were treated first with platelet extract containing PDGF and then with 5% plasma containing methotrexate as above; 18 h later, when 90% of the cells were growth arrested in early S phase, the methotrexate block was removed. ^{3}H-TdR was added to the cultures 16 h after removal of the methotrexate to study DNA synthesis in the post-mitotic daughter cells. Without further PDGF treatment, <10% of the daughter cells entered the S phase within the 27 h period after removal of the methotrexate block (Table 1). Addition of partially purified PDGF to the parent cells for 2-h periods during the first 8 h after removal of the methotrexate (corresponding largely to parental cell S and G_2) induced 60–70% of the daughter cells to synthesise DNA (Table 1). The

Table 1 Treatment with PDGF during the S phase stimulates post-mitotic daughter cells to synthesise DNA

Interval (h) treated with PDGF after methotrexate removal	% Of daughter cells synthesising DNA
0–2	67
2–4	68
4–6	70
6–8	59
Untreated	9

Density-inhibited BALB/c 3T3 cells were treated with 150 μg ml^{-1} of heat-treated platelet extract containing PDGF for 5 h, washed once[2] and transferred to medium containing 5% (v/v) dialysed human plasma containing 106 nM methotrexate. After 18 h all cultures were transferred to fresh medium containing 5% plasma, 100 nM hypoxanthine and 16 nM thymidine. Addition of ^{3}H-TdR (5 μCi ml^{-1}, final concentration 0.3 Ci mmol^{-1}) from 0–2 h after the methotrexate was removed, followed by immediate fixation and autoradiography, demonstrated that 90% of the parent cells synthesised DNA. Duplicate cultures were treated with heat-treated platelet extract (150 μg ml^{-1}) in the presence of hypoxanthine and thymidine, but in the absence of plasma for the interval shown (or were left untreated), washed twice with medium and returned to the plasma-supplemented medium containing hypoxanthine and thymidine. Approximately 16 h after the methotrexate was removed, ^{3}H-TdR was added; the cultures were fixed and processed for autoradiography 11 h later and the percentage of post-mitotic daughter cells entering the S phase was determined.

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percentage of PDGF-treated daughter cells that entered the S phase was also controlled by the concentration of plasma in the medium; in 0.25% plasma 30% of the daughter cells entered the S phase, compared with 90% in 5% plasma.

To study the effect of the PDGF concentration on the percentage of post-mitotic daughter cells stimulated to enter the S phase, platelet extract was briefly added to parental cells undergoing DNA synthesis. The percentage of daughter cells stimulated to synthesise DNA increased approximately linearly as a function of the logarithm of the platelet extract concentration over a 10-fold range (Fig. 2*a*). Treatment of parent cells with pure PDGF[19] also stimulated the daughter cells to synthesise DNA in a dose-dependent fashion (Fig. 2*b*). A 2-h treatment with 5 ng ml^{-1} of a homogeneous preparation of PDGF stimulated 50% of the daughter cells to enter the S phase.

To study the lag phase until DNA synthesis in post-mitotic daughter cells, duplicate cultures were released from the methotrexate block and briefly treated with partially purified PDGF. Treatment of the parent cells with PDGF from 0–2 or 4–6 h after release of the methotrexate block (corresponding to early or middle S phase) induced the daughter cells to begin synthesising DNA 15 h from the release of the methotrexate block (Fig. 3*a*, *b*), approximately 6 h after the beginning of mitosis. Addition of PDGF from 8–10 h after methotrexate removal (corresponding largely to parental G_2 and M phases) stimulated the daughter cells to enter the S phase at 17 h, approximately 7 h after the PDGF treatment was completed and the cells were returned to plasma (Fig. 3*c*). Addition of PDGF to the post-mitotic daughter cells, 12–14 h after the methotrexate was removed largely corresponding to G_1, 3–5 h after the beginning of mitosis, induced the cells to enter the S phase 7 h after the PDGF treatment (Fig. 3*d*). However, addition of the PDGF to the post-mitotic daughter cells 16–18 h after methotrexate was removed (7–9 h after the beginning of mitosis) resulted in a significant delay in S phase entry (Fig. 3*e*). These cells began to enter the S phase 12 h after PDGF was removed, demonstrating that they had become arrested in the G_0 phase of the cell cycle.

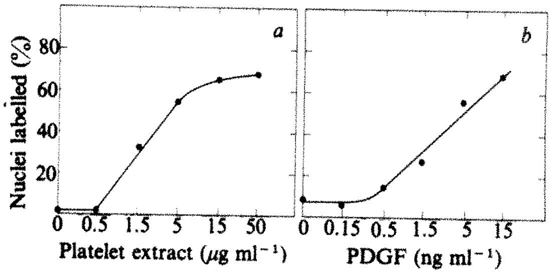

Fig. 2 The concentration of PDGF regulates the percentage of post-mitotic daughter cells synthesising DNA. Density-inhibited BALB/c 3T3 cells were treated with partially purified PDGF and transferred to 5% plasma-supplemented medium containing methotrexate as outlined in Fig. 1 legend. After 18 h the medium was removed from all cultures; some cultures were transferred to medium containing 5% plasma, hypoxanthine and ^{3}H-TdR (5 μCi ml^{-1}; 0.3 Ci mmol^{-1}) and fixed 8 h later. Autoradiography demonstrated that 90% of the PDGF-stimulated cells and <1% of the unstimulated cells entered the S phase. Duplicate cultures were treated with heat-treated platelet extract (*a*), or pure PDGF which was purified to homogeneity from clinically outdated human platelets as has been described elsewhere[19] (*b*), at the concentrations shown, plus thymidine and hypoxanthine. Two hours later the PDGF source was removed, the cultures were washed and transferred to 5% plasma-supplemented medium containing hypoxanthine and thymidine. ^{3}H-TdR was added to the cultures 10 h after removal of the platelet extracts. The cultures were fixed and processed for autoradiography 16 h later. The lag phase for DNA synthesis is 12 h after plasma addition for the G_0-arrested cultures[2], and 13 h for the second wave of DNA synthesis (Fig. 3*a*) in the S phase cultures.

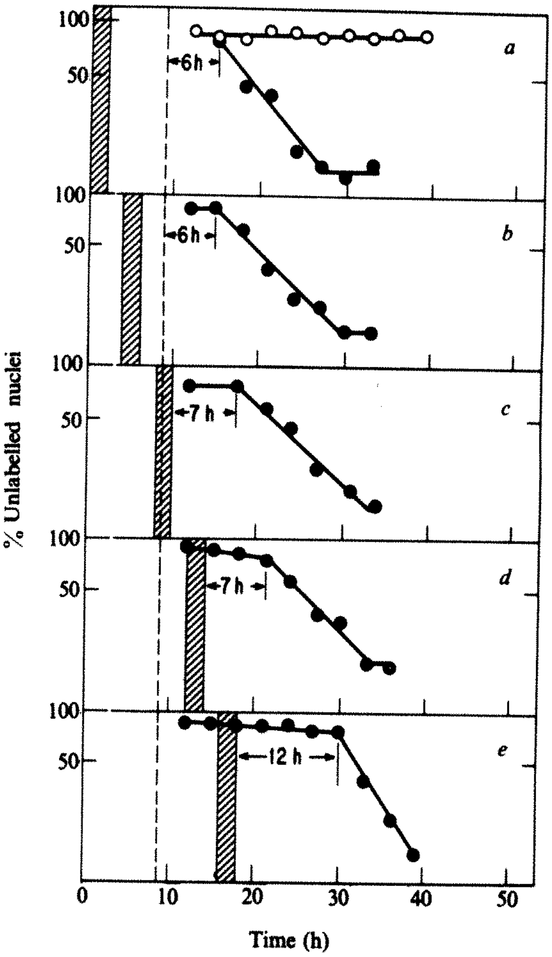

Fig. 3 Treatment with PDGF during S, G_2 or M phases, or immediately after mitosis, prevents replicating BALB/c 3T3 cells from becoming growth arrested at G_0. Density inhibited BALB/c 3T3 cells were treated with partially purified PDGF and transferred to 5% plasma-supplemented medium containing methotrexate as outlined in Fig. 1 legend. After 18 h, the medium was removed from all cultures; some cultures were transferred to medium containing 5% plasma, hypoxanthine and ^{3}H-TdR (5 μCi ml^{-1}; 0.3 Ci mmol^{-1}) and fixed 8 h later. Autoradiography demonstrated that 91% of the PDGF-stimulated parent cells entered the S phase. Duplicate cultures were transferred to 5% platelet-poor plasma containing hypoxanthine and thymidine. Some cultures (●) were treated with platelet extract (150 μg ml^{-1}) containing partially purified PDGF and hypoxanthine and thymidine for 2-h periods at the times indicated by the shaded bars or were left untreated (○). ^{3}H-TdR (5 μCi ml^{-1}; final concentration 0.3 Ci mmol^{-1}) was added to these cultures 10 h after the removal of the methotrexate. The cells were fixed at the indicated times and processed for autoradiography. The data are plotted according to the method of Smith and Martin[24]. The dashed line is shown to mark the beginning of mitosis (see Fig. 1).

The present data clarify the role of PDGF in regulating fibroblast growth by demonstrating that this hormone has two activities. PDGF can act during the late stages of the cell cycle, or shortly after mitosis, to prevent BALB/c 3T3 cells from entering G_0. It can also act on G_0-arrested cells to promote exit from the quiescent state. A brief treatment of quiescent cells with PDGF stimulates a single replicative cycle.

Treatment of parental cells with PDGF, during or after the S phase, allows the daughter cells to synthesise DNA. One possibility is that PDGF stimulates 'G_0-specific' events concomitantly with S, G_2 and M phases. In such a case, G_0 might not represent a true growth arrest state, but would merely reflect a series of time-dependent events which must occur in all cell cycles before DNA synthesis. The increased lag period noted in post-mitotic quiescent cells would represent the time required to complete these events. However, the addition of PDGF to the daughter cells immediately after parental mitosis also prevents growth arrest, with cells entering the S phase after a short lag period (Fig. 3*d*). Further delay in the addition of PDGF causes cells to become quiescent (Fig. 3*e*). Thus, cells enter G_0 if they are not treated with PDGF before a critical period in the replicative cycle, approximately 3–5 h after the beginning of mitosis. Addition of PDGF after this period does not prevent entry into G_0, but releases cells from G_0, a process which requires an additional 5 h before entry into the S phase. Clearly, PDGF does not simply cause 'G_0-specific' events to be telescoped into the preceding cell cycle. The evidence supports the concept that G_0 is a distinct growth arrest state which cells can enter some time after mitosis.

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Metabolites of diethylstilboestrol induce sister chromatid exchange in human cultured fibroblasts

H. W. Rüdiger*, F. Haenisch*, M. Metzler†, F. Oesch§ & H. R. Glatt‡

* Universitäts-Krankenhaus, Eppendorf, Martinistrasse 52, D-2000 Hamburg 20, FRG
† Institute of Toxicology of the University, Versbacher Landstrasse 9, D-8700 Würzburg, FRG
‡ Institute of Pharmacology, University of Mainz, Obere Zahlbacher Strasse 67, D-6500 Mainz, FRG

Diethylstilboestrol (DES) is one of the few substances for which a clear association with carcinogenicity has been established in man[1,2]. Nevertheless, it is still widely used, mainly as a cheap oestrogen to increase the slaughter weight of beef[3], but in spite of this it is not known if residues in the meat or metabolites excreted by the cattle are hazardous to man. It is also unknown whether there is a threshold dose below which DES is harmless. A threshold might be expected if a hormonal mechanism of carcinogenesis rather than metabolic activation to an electrophilically reactive species operates. This possibility was supported by the observations that DES, in contrast to most other carcinogens, failed to induce mutations in the *Salmonella*/microsome test[4–6] or malignant transformations of eukaryotic cells in culture[7]. It is also disturbing that DES, one of the few known human carcinogens was negative in these two most widely used short-term tests introduced as fast early-warning systems for potential carcinogens. We now report that DES is positive in sister chromatid exchange (SCE) induction, a short-term test for which a high correlation with the carcinogenicity of the compounds tested has been observed[8–10]. Moreover, we show that metabolic activation was involved. Two different pathways leading to metabolites much more active in SCE induction than DES itself ('proximate agents') were established.

DES-α, β-oxide and β-dienoestrol (Fig. 1) were synthesised as described previously[11]. The mutagenicity test was performed as described by Ames[12]. As mammalian metabolising system S-9 mix[12] from liver homogenate of Aroclor 1254-treated male Sprague–Dawley rats (200–300 g) was used. Fibroblast cultures were established[13] from skin biopsies of the inner side of the upper arm of healthy male and female volunteers of Caucasian origin. 5-Bromodeoxyuridine (30 μg ml^{-1}) and the test compound were incubated with the cells for 72 h. After staining with acridine orange[14], SCE was directly evaluated under a fluorescent microscope.

The initial experiment revealed that DES induces SCE. The number of SCEs was increased even at low doses with a further shallow increase as the DES concentration is raised (Fig. 2, open symbols). The results obtained with diploid fibroblast cultures of five different donors were very similar. Interestingly, lower concentrations of DES were sufficient to cause SCE than of the positive control benzo (*a*) pyrene (Table 1). To clarify whether DES induces SCE itself or whether it requires metabolic activation, we used two methods—inhibition of metabolism and test of metabolites for induction of SCE. Several pathways of DES metabolism are known[11,15]. All include some chemically reactive intermediates: (1) hydroxylation *ortho* to the phenolic hydroxyl groups leads to highly reactive catechols; (2) oxidation of the olefinic α, β-double bond produces an electrophilically reactive epoxide as the primary metabolite; (3) β-dienoestrol, a quantitatively important metabolite is formed by peroxidases, probably via highly reactive semiquinones and quinones. β-Dienoestrol can be further oxidised to ω-hydroxy-β-dienoestrol which can form reactive esters. At first, we tested two key metabolites, DES-α,β-oxide and β-dienoestrol (structures shown in Fig. 1). We observed an increase of SCE at even lower concentrations of test compound than with DES (Fig. 2). Dual reciprocal plots of the SCE induced against the concentration of

Table 1 SCE-induction by DES and metabolic intermediates

Test compound	Maximum SCE per metaphase	Concentration for half maximal induction for SCE*	Potency†
DES	11.0	133 nM	1
DES-α, β-oxide	18.2	40 nM	9.6
β-Dienoestrol	16.8	5 nM	70
Benzo(*a*)pyrene	15.7	2,100 nM	0.14

SCEs were investigated as described in Fig. 2 legend. Controls showed 7.2 ± 0.9 (s.d.) SCE per metaphase.

* Values are calculated from dual reciprocal plots by linear regression analysis. The *r*-values were >0.97.

† The maximal number of SCE which were caused by the test compound (minus the number of spontaneous SCE) was divided by the concentration of test compound which caused the half-maximal effect. This value was divided by that of DES and is used as measure of the ability of the compound to cause SCE.

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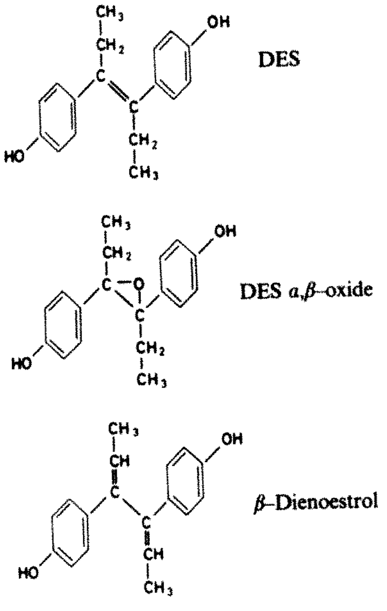

Fig. 1 Chemical structure of diethylstilboestrol (DES), and the two metabolites used for SCE induction.

inducing substance were used to calculate the maximum frequency of SCE and the concentrations for half maximal SCE induction (Table 1). The much higher potency of the metabolites strongly suggests that the induction of SCE by DES requires a metabolic activation. As we had previously shown[16] that the metabolic activation of benzo(*a*)pyrene to a derivative inducing SCE can be inhibited by α-naphthoflavone, which inhibits mono-oxygenases[17], we determined the SCE induction by DES and two metabolites in the presence of α-naphthoflavone (Fig. 3). The induction of SCE normally observed with DES was completely inhibited by α-naphthoflavone and was markedly reduced when α-naphthoflavone was added together with β-dienoestrol. The SCE-induction obtained with DES-α, β-oxide, however, could only be lowered about 35% by the presence of α-naphthoflavone. This provides additional evidence that DES and also β-dienoestrol require metabolic activation by mono-oxygenase for the induction of SCE.

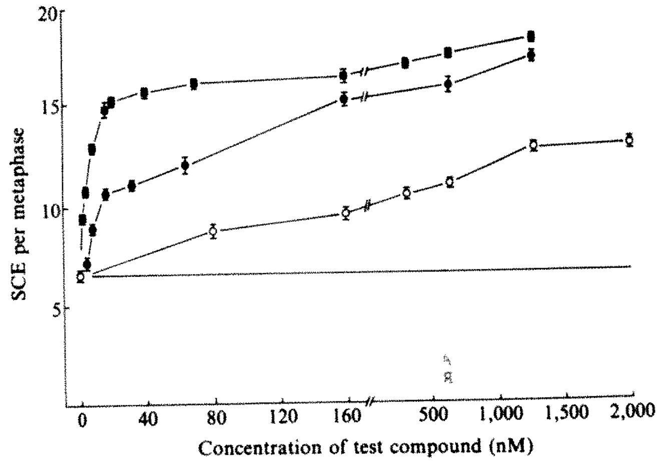

Fig. 2 SCE induction by DES (○) DES-α, β-oxide (●) and β-dienoestrol (■). The test compounds were dissolved in ethanol and then mixed with a twofold excess of sterile fetal bovine serum (FBS). Various amounts of these stock solutions (which were at least 100-fold concentrated as compared to the final dilution in the culture medium) were added to 25 cm^2 cell culture flasks (Falcon) containing 5×10^5 cells together with 5 ml Dulbecco's modified Eagles medium (GIBCO), supplemented with 10% FBS (GIBCO), 2×10^5 IU l^{-1} penicillin, 0.1 g l^{-1} neomycin and 0.1 g l^{-1} streptomycin, and buffered with 20 mM HEPES and 0.5 g l^{-1} $NaHCO_3$. The concentration of ethanol in the culture medium did not exceed 0.5%. Controls received 0.5% ethanol only.

Recently it was reported that DES can induce unscheduled DNA synthesis[18] in HeLa cells, and induces mutations[19] in a mouse lymphoma cell line. However, both effects could only be demonstrated over a very narrow, already markedly cytotoxic dose range and quantitative reproducibility was poor. Thus, neither test is suitable for studying factors which determine susceptibility towards DES. In contrast, SCE induction by DES in human diploid fibroblasts shown in this study did occur at very low doses and was dose-dependent over a wide range of concentrations. Thus the system could be used to investigate whether metabolic activation was involved, and to discover pathways leading to metabolities more active in SCE induction than DES itself ('proximate agents').

Table 2 Reversion of *his*⁻ *S. typhimurium* to histidine prototrophy

		his⁺ colonies per plate (range)			
Test compound (μg per plate)	S-9 mix	TA 100	TA 1535	TA 1537	TA 98
Solvent control	+	100–134	9–20	17–31	31–39
3.15–3000 DES	+	95–170	6–22	13–33	25–54
3.15–3000 DES α, β-oxide	+	89–141	7–24	5–24	23–41
3.15–315 β-Dienoestrol	+	85–149	13–19	10–27	34–63
10 Benzo(*a*)pyrene	+	980–1720	31–44	152–274	589–1,130
10 2-Aminoanthracene	+	5,500–9,100	568–668	240–318	6,200–6,700
90 3-Methylcholanthrene	+	5,000–8,900	12–23	50–97	1,180–1,520
Solvent control	−	153–192	17–30	8–13	11–21
3.15–315 DES	−	126–165	12–20	1–11	14–22
3.15–3000 DES α, β-oxide	−	144–200	8–22	5–15	15–24
3.15–315 β-Dienoestrol	−	139–189	13–23	4–7	11–21
1 Benzo(*a*)pyrene 4, 5-oxide	−	2,340–4,000	21–31	620–700	5,000–5,700
10 *N*-Methyl-*N*′-nitro-*N*-nitrosoguanidine	−	50,000–61,000	45,000–80,000	68–115	38–41

Histidine-dependent bacteria (*S. typhimurium* strain TA 100, TA 1535, TA 1537 or TA 98), test compound (dissolved in 10-30 μl dimethyl sulphoxide with or without a mammalian metabolising system (S-9 mix from liver homogenate of Aroclor 1254-treated rats) were poured with a histidine-poor top agar on minimal agar plates. Colonies (*his*⁺ revertants) were counted after incubation for 3 d at 37 °C. DES and its metabolites were tested in duplicates at the following doses: 3.15; 10; 31.5; 100; 315; 1,000 and 3,000 μg per plate. The table shows the range of the number of colonies at those doses where neither a significant reduction of the number of *his*⁺ colonies nor of the *his*⁻ background lawn was observed. Each individual experiment with S-9 mix contained benzo(*a*)pyrene, 2-aminoanthracene, 3-methylcholanthrene and solvent controls. Each experiment for direct mutagenicity contained benzo(*a*)pyrene 4, 5-oxide, *N*-Methyl-*N*′-nitro-*N*-nitrosoguanidine and solvent controls. Values for these controls represent ranges of all the experiments used for this study.

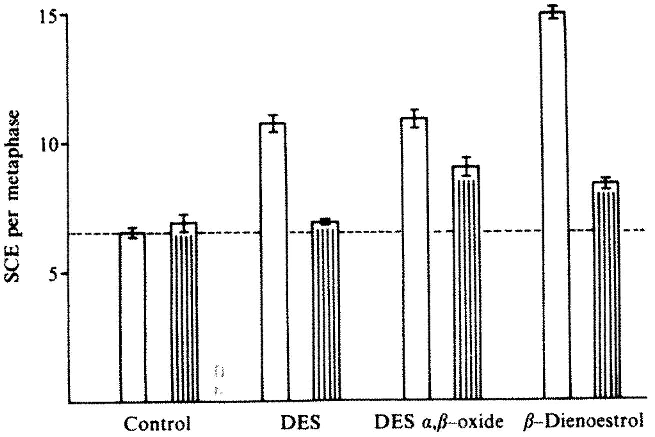

Fig. 3 Effect of α-naphthoflavone on the induction of SCE by DES, DES-α, β-oxide and β-dienoestrol. The experiments were performed as described in Fig. 2. α-Naphthoflavone was dissolved in acetone, mixed with a 40-fold excess of FBS and added to the culture. DES was present at 1.25 μM, DES-α, β-oxide and β-dienoestrol at 20 nM. The bars show mean numbers of SCE per metaphase ±s.d. of incubations without (☐) and with (▥) α-naphthoflavone (5 $\mu g\ ml^{-1}$).

Note that the concentrations of test compound at which SCE were induced were low not only in absolute terms but also in relation to the toxicity of the compounds. With DES-α, β-oxide and β-dienoestrol cytotoxic effects that lead to an altered microscopic appearance of the cells and to a reduced number of metaphases were observed only at concentrations more than 100-fold greater than those needed for a marked SCE induction. The induction of SCE at doses which are much lower than those needed for observing toxicity favour the assumption that the exchanges of DNA fragments observed as SCE reflect specific DNA-directed damage rather than DNA alterations as an effect of broad disturbances of cell growth.

These results showed that some metabolites of DES are much more potent inducers of SCE than DES itself, so we investigated if these metabolites could induce mutations in *Salmonella typhimurium* either directly or after activation by mammalian tissue preparations. Table 2 shows that neither DES nor the two metabolites DES-α, β-oxide and β-dienoestrol induced any mutations to histidine prototrophy with *his* *S. typhimurium* strains in conditions where *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine and benzo(*a*)pyrene-4,5-oxide (in the experiments for direct mutagenicity) and benzo(*a*)pyrene, 3-methylcholanthrene and 2-aminoanthracene (in the presence of S-9 mix) strongly increased the number of his^+ revertant colonies.

Our results show that from DES at least two metabolic routes lead to metabolites which cause SCE: (1) via or to DES-α, β-oxide; (2) via β-dienoestrol which is further activated by monooxygenase. The activation via β-dienoestrol led to much stronger SCE effects than the activation via DES-α, β-oxide. In this context it is interesting that organs susceptible to DES carcinogenesis possess high activities of peroxidase[20], which metabolises DES to β-dienoestrol. The demonstration of metabolic activation of DES for SCE induction does, of course, not exclude that hormonal mechanisms and stimulation of cell proliferation may be important contributors to its carcinogenic effects.

The reasons why results with SCE are not reproduced in the Ames test are not clear. It may be that the *S. typhimurium* assay cannot detect mutations caused by DES metabolites as it is a back-mutation assay or because the exchange of identical genetic material during the SCE may not lead to mutation. Alternatively, metabolic factors may be involved—for example, enzymes (such as conjugating enzymes) may be required for the activation of DES which are active in human fibroblasts but not in the dilute postmitochondrial liver fraction in the plates of the bacterial mutagenicity test. Or conversely, enzymes may be present in the S-9 mix which efficiently inactivate the active DES-metabolites.

The induction of SCE in fibroblasts by DES is an *in vitro* system which will be of value as a tool for the study of the mechanisms of activation and inactivation of DES and the reasons why it is inactive in other *in vitro* systems and in many organs *in vivo*[3,21].

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Note added in proof: On 28 June 1979 the FDA banned the use of DES as a growth promoter in cattle and sheep.

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Identical 3′ non-coding sequences in five mouse Ig κ chain mRNAs favour a unique C_κ gene

Nicholas M. Gough, Suzanne Cory & Jerry M. Adams

Molecular Biology Laboratory, The Walter and Eliza Hall Institute of Medical Research, PO Royal Melbourne Hospital, Victoria 3050, Australia

The constant regions of immunoglobulin κ chains are encoded by a very small but unknown number of genes. The kinetics of nucleic acid reassociation is consistent with up to four C_κ genes per haploid mouse genome[1–4], while saturation hybridisation suggests that there are two C_κ genes[5]. The amino acid sequences proposed for seven κ C regions do differ slightly[6–10], but at least some of these differences result from sequencing errors[11]. Determining the nucleotide sequence of the C region of κ mRNAs[11] and the adjacent 3′ non-coding region should more readily reveal any slight differences reflecting a multiplicity of C_κ genes. Examination of the 3′ non-coding region may be particularly useful, as this region is not constrained to encode the largely invariant C_κ amino acid sequence. Sequences reported for the 3′ terminal regions of two κ mRNAs, MOPC 21 (refs 11, 12) and MOPC 41A (ref. 13), were identical for the first 59 residues, but the subsequent, tentative, 45 residues proposed for MOPC 41A mRNA differed from the MOPC 21 sequence. It was unclear, however, whether these differences resulted from methodological problems, or genuine sequence divergence. We have therefore re-examined the MOPC 41A 3′ terminal sequence in a direct comparison with that of MOPC 21 and also determined 3′ terminal sequences of about 100 residues in three other κ mRNAs. We confirm here the sequence presented by Hamlyn *et al.*[11] for the MOPC 21 mRNA and show that not only the MOPC 41A mRNA, but also the three other κ mRNAs have the same sequence.

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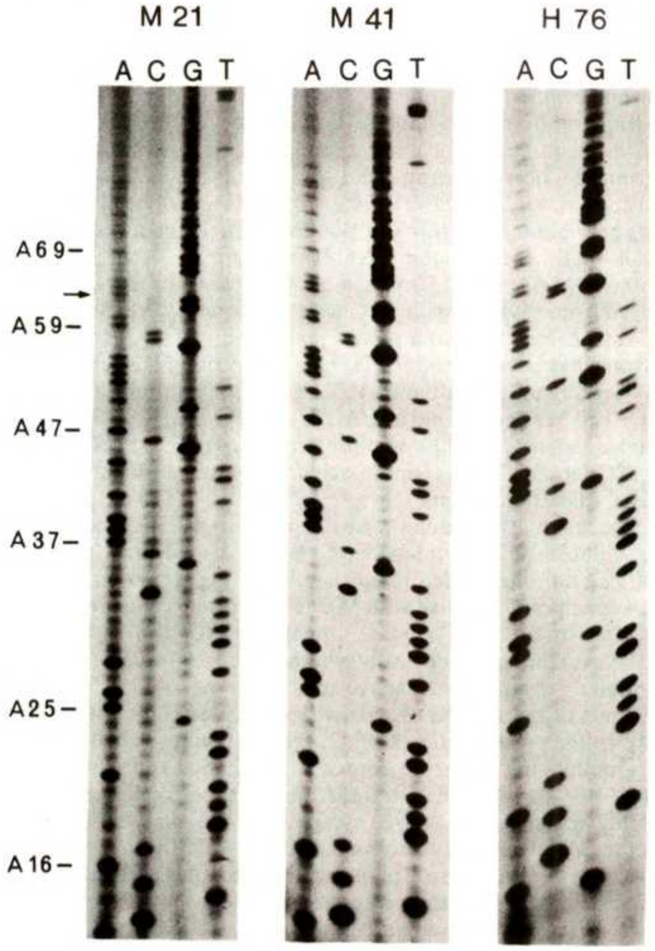

Fig. 1 Polyacrylamide gels used to determine sequences complementary to the 3′ termini of MOPC 21, MOPC 41A and HPC 76 κ mRNAs. The oligonucleotide d(pT_{10}-C-A) was used as primer, in a fourfold molar excess over the mRNA templates which were isolated as described previously[24,25]. cDNA was synthesised in 6-μl reactions containing 50 mM Tris-Cl *p*H 8, 50 mM KCl, 8 mM $MgCl_2$, 10 mM dithiothreitol, 2 μM [$\alpha-{}^{32}P$]dATP and about 2 units of reverse transcriptase. Dideoxynucleotide triphosphates (P-L Biochemicals) and the corresponding dNTPs were in the following molar ratios: ddATP:dATP, 2.7:1; ddCTP:dCTP, 1.3:1; ddGTP:dGTP, 0.6:1; ddTTP:dTTP, 0.7:1. Incubation was at 39 °C for 15 min, followed by a 'chase' reaction containing 200 μM of each of the four dNTPs (39 °C, 15 min). Samples were denatured in formamide and loaded onto a 0.35-mm thick 12% polyacrylamide gel[26] containing 7 M urea, 90 mM Tris-borate, 2.5 mM EDTA. Electrophoresis was for 2.5 h at 30 mA and autoradiography at −80 °C. The arrow indicates a position at which Hamlyn *et al.*[11] have suggested a correction to their previously published sequence for the MOPC 21 κ mRNA[12], the elimination of a C residue; our results confirm this correction.

Sequences were derived by an adaptation[14,15] of the dideoxynucleotide procedure for sequencing DNA templates[16]. Complementary DNA was transcribed using the oligonucleotide d(pT_{10}-C-A)[12] as primer. Figure 1 shows examples of autoradiographs of polyacrylamide gels used to determine sequences complementary to the 3′ terminal regions of three κ mRNAs. Clearly, the patterns of bands shown are identical. These gels allowed sequences of about 60–70 nucleotides to be determined. By manipulating the concentrations of the chain-terminating dideoxynucleotides and the time of electrophoresis, sequences of over 100 residues could be obtained. The sequences derived for the five mRNAs examined, and for MOPC 21 (ref. 11), are shown in Fig. 2. At no position did any of the sequences differ distinctly from that of MOPC 21. There were a few positions towards the ends of the sequences where we could not clearly distinguish between two nucleotides, but in each case, one of the choices was that found in the MOPC 21 sequence.

The known 3′ terminal sequences in mammalian mRNAs show considerable differences[11,17–21]. Even between the mRNAs encoding the closely related human and rabbit α-globins and human, rabbit and mouse β-globins there are distinct sequence and length differences within this region[17–20]. It has been suggested that these regions may not have a precise sequence-dependent function[21]. The apparent identity of the five κ non-coding regions is therefore unlikely to result merely from strong evolutionary selection for a specific sequence, but rather favours the suggestion that there is only one mouse C_κ gene. If there were two C_κ genes which differed in the region examined and were expressed with equal frequency, the chance that five sequences would be of one type is 1 in 16; similarly, for three C_κ genes, the chance would be 1 in 81. Naturally, our results would say little about the possibility of a C_κ gene expressed infrequently in myelomas.

Nucleotide sequences reported for a substantial portion of the C region in two κ mRNAs (MOPC 21 (ref. 11) and MOPC 149 (ref. 22)) are identical and contain no 'silent' base changes, providing further evidence in favour of a unique C_κ sequence. Similarly, the detailed restriction endonuclease cleavage maps of seven different cloned κ mRNA sequences are identical throughout the *C* region (N.M.G., E. Webb and J.M.A., in preparation).

It seems remarkable that no differences have been detected in the C_κ regions examined, as the plasmacytomas from which they were isolated are of independent neoplastic origin and have been serially transplanted for many years. This suggests that if there is a somatic mechanism for generating V region diversity[23], it must either be directed specifically to the V region genes, or no longer be operational within the terminally differentiated plasma cell.

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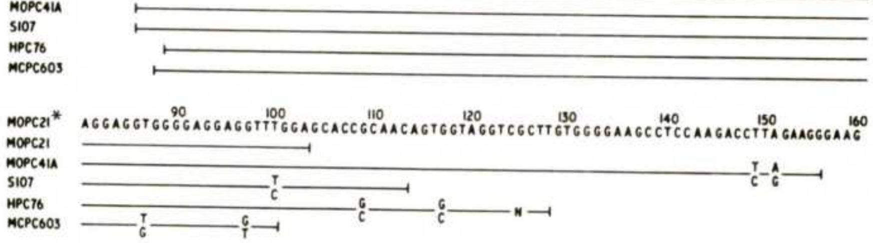
MOPC21*
1 10 20 30 40 50 60 70 80
CAAGTGCAAAGACTCACTTTATTGAATATTTTCTGCAAATATTAGCATGATAAAAGCCAAGGAAAGGGAGGAGGAGGAGA
MOPC21
MOPC41A
S107
HPC76
MCPC603
MOPC21*
90 100 110 120 130 140 150 160
AGGAGGTGGGGAGGAGGTTTGGAGCACCGCAACAGTGGTAGGTCGCTTGTGGGGAAGCCTCCAAGACCTTAGAAGGGAAG
MOPC21
MOPC41A
T C A G
S107
T C
HPC76
G C G C N
MCPC603
T G G T

Fig. 2 DNA sequence complementary to the 3′ non-coding region of five κ light chain mRNAs. The mRNAs were from the tumours MOPC 41A, McPC 603, S107 (reviewed in ref. 27), HPC 76 and from the P3 cell line[28] (derived from the tumour MOPC 21). The MOPC 21 κ sequence (*) is taken from the complete sequence of Hamlyn *et al.*[11]. The solid lines indicate the regions examined in the other mRNAs which had sequences clearly identical to that of MOPC 21. Bands were usually not detected at positions corresponding to the first few residues after the primer, as is often found with this technique. At positions where there was ambiguity the possible residues are given. In the HPC 76 sequence there was one position (N) where a band occurred in the equivalent position in all tracks. The arrow indicates the position at which a C residue had previously been assigned[12] (see Fig. 1).

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A normal cell protein cross-reactive to the major Abelson murine leukaemia virus gene product

Owen N. Witte, Naomi E. Rosenberg* & David Baltimore

Department of Biology and Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139 and *Cancer Research Center, Tufts University School of Medicine, Boston, Massachusetts 02111

Abelson murine leukaemia virus (A-MuLV) is a replication-defective retrovirus capable of rapid *in vivo* and *in vitro* transformation of bone marrow lymphoid target cells. A-MuLV was isolated from a steroid treated BALB/c mouse inoculated with the replication competent Moloney-MuLV (M-MuLV)[1–4]. The A-MuLV genome (5.5 kilobases) retains regions of precise homology to M-MuLV at its 5′ end (1,320 bases) and 3′ end (730 bases). The centre of the A-MuLV genome (3,500 bases) is non-homologous to M-MuLV and presumably represents unique sequences derived from the mouse genome by a recombinational event[5]. The only known protein encoded by the A-MuLV genome is one of MW 120,000, called P120 (refs 6, 7). About one-quarter of this protein is encoded by the 5′-terminal M-MuLV-related sequences and the rest is encoded by the A-MuLV unique sequences. P120 has been found in all cell lines transformed by the strain of A-MuLV that originally derived from ANN-1 cells[2], including transformed non-producer cells. P120 can be translated *in vitro* using A-MuLV genomic RNA as messenger RNA. P120 is a phosphoprotein but there is no evidence for glycosylation[6,8,9]. We have used an anti-A-MuLV syngeneic tumour regressor serum with defined specificities to search for cross-reacting normal cellular proteins. We have identified a protein of MW 150,000 (NCP150) in metabolically labelled thymus and other lymphoid organs. NCP150 by absorption and competition analysis is closely related to the unique region of the A-MuLV P120 protein.

Mouse syngeneic tumour regressor antiserum with high reactivity to the A-MuLV unique region of P120 was prepared. After absorption to remove M-MuLV protein reactivity this antiserum contains residual activity for P120 demonstrated by direct immunoprecipitation and tumour cell surface immunofluorescence[9]. This A-MuLV tumour regressor serum (anti-AbT serum) was used to search by immunoprecipitation for normal cellular proteins.

Explants of mouse lymphoid tissues were labelled for 1 h with ^{35}S-methionine, extracted, immunoprecipitated with anti-AbT serum or normal serum and examined by electrophoresis through an SDS-polyacrylamide gel. A protein of MW 150,000 was precipitated by anti-AbT but not normal serum (Fig. 1); we shall call the protein NCP150 (normal cell protein, 150,000 MW). NCP150 was most prominent in extracts prepared from thymus tissue but smaller amounts were detectable in spleen and bone marrow. Precipitation of NCP150 from thymus has been reproducible; no other protein has reproducibly been reactive with anti-AbT serum but not normal serum. NCP150 has been found in BALB/c, C57BL/6, C57L and SJL mouse strains. No additional proteins were found if ^{3}H-leucine or ^{14}C-labelled mixed amino acids were used to label thymus tissue (data not shown).

The reactivity of our anti-AbT serum with NCP150 could not be blocked by unlabelled M-MuLV virion proteins ($100\ \mu g\ ml^{-1}$) (included in all samples in Fig. 1) nor could any sera specifically reactive with M-MuLV proteins encoded in the *gag*, *pol* or *env* gene regions[10] precipitate NCP150.

To examine more critically whether NCP150 was being precipitated by antibody reactive to the unique A-MuLV determinants of the P120 molecule, we performed competition and absorption tests. Extracts of C57BL/6 thymus labelled with ^{35}S-methionine were the source of NCP150, and extracts of 2M3 cells—an A-MuLV-transformed lymphoid cell line—were used as a comparison source of labelled P120. Anti-AbT serum in the presence of excess unlabelled M-MuLV virion proteins was used to precipitate all experimental samples and unlabelled cell extracts were used as competitor.

Normal mouse serum precipitated no NCP150 or P120 (Fig. 2, lanes *a*) and anti-AbT serum precipitated readily detectable bands of NCP150 and P120 (Fig. 2, lanes *b*). The amounts of anti-AbT serum used had been titrated to be limiting in the precipitation. When increasing amounts of unlabelled extracts of an A-MuLV-transformed non-producer NIH/3T3 cell line were included, immunoprecipitation of both NCP150 and P120 was blocked (Fig. 2, lanes *c–e*). Equivalent amounts of extract from a Moloney sarcoma virus transformed non-producer NIH/3T3 cell line did not block the specific precipitations (Fig. 2, lanes *f–h*). Extracts of NIH/3T3 cells infected with the spleen focus-forming component of the Friend mouse leukaemia virus also failed to block precipitation (data not shown). Thus competition experiments show that all of the reactivity of anti-AbT serum with NCP150 can be blocked by extracts containing P120 (A-MuLV transformed cells) and not by extracts of transformed cells that lack P120 (Moloney sarcoma virus transformed cells).

As another specificity test, we pre-absorbed anti-AbT serum with intact, viable A-MuLV-transformed non-producer NIH/3T3 cells to remove reactivity to the P120 determinants expressed on the cell surface. Such absorptions have been shown[9] to remove the ability of anti-AbT serum to stain the cell membrane of A-MuLV transformed cells; it also markedly reduced the ability of the serum to immunoprecipitate P120. Figure 2, lanes *i*, shows that precipitation of NCP150 and P120 with unabsorbed control serum was evident but lanes *j* show a markedly reduced precipitation with the serum preabsorbed by live cells. Thus, for selected batches of anti-AbT serum, most of the reactivity is with cell surface-exposed regions of P120 and these regions are also those by which NCP150 is precipitated. Whether these regions of NCP150 are cell surface-exposed remains to be determined.

These results demonstrate that NCP150 and the unique region of P120 share common antigenic determinants. The precise arrangement of these antigenic sites and molecular extent of homology between NCP150 and P120 cannot be determined using these techniques. Further work involving

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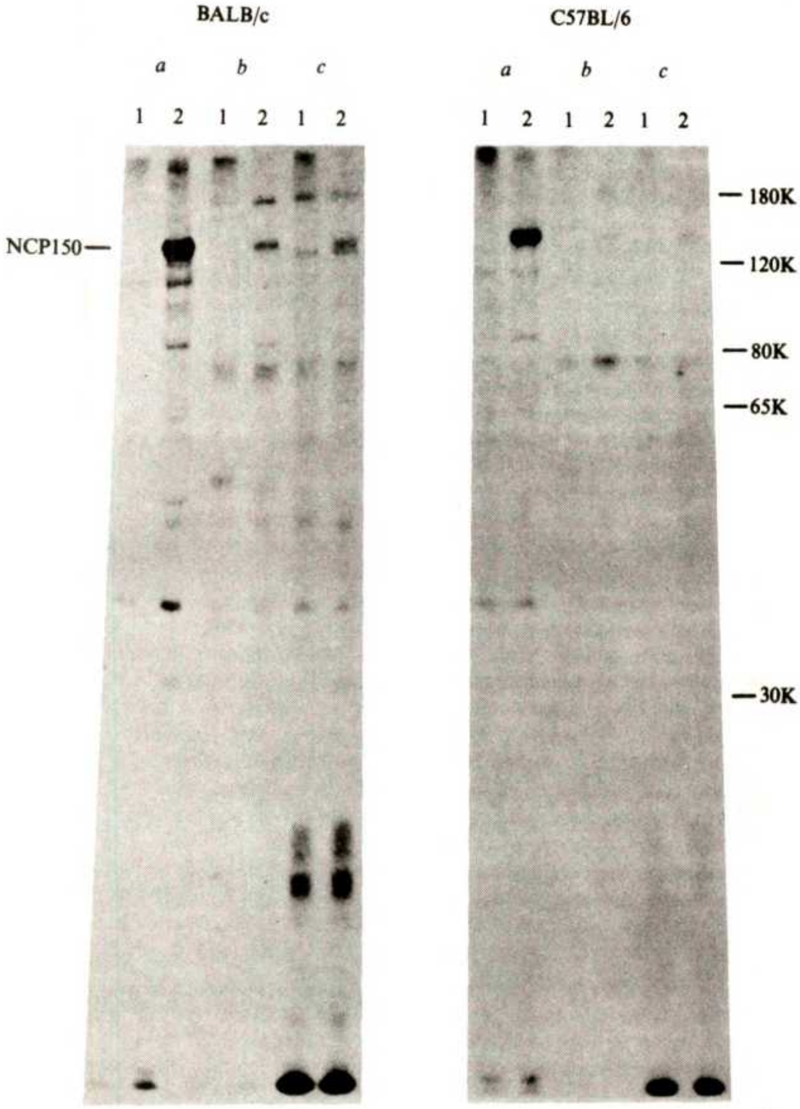

Fig. 1 Immunoprecipitation of a normal cellular protein with anti-Abelson MuLV tumour regressor serum. *In vitro* explants prepared as single cell suspensions (2×10^7 nucleated cells) of thymus (*a*), spleen (*b*) or bone marrow (*c*) from normal 5-week-old BALB/c or C57BL/6 mice were labelled with 100 μCi of ^{35}S-methionine (NEN) in 2 ml of methionine free media with 10% dialysed fetal calf serum for 1 h at 37 °C. Cells were pelleted, extracted and clarified for immunoprecipitation as previously described[6] at 10^7 cells per ml with the inclusion of the protease inhibitor phenylmethylsulphonylfluoride at 2 mM. Aliquots of 1 ml were reacted (16 h at 4 °C) with 5 μl of either normal mouse serum (lane 1) or anti-Abelson MuLV syngeneic tumour regressor serum (anti-AbT, lane 2). All tubes contained 100 μg ml^{-1} unlabelled virion proteins of Moloney MuLV. Immunoprecipitates were collected with *Staphylococcus aureus* and analysed on a SDS–10% polyacrylamide gel developed by fluorography as previously described[10]. The migration position of molecular weight standards given is shown on the right hand side.

formal peptide map comparison will be required to answer such questions.

The complete spectrum of cell types that contain NCP150 and the relative concentrations of the protein in these cells have yet to be ascertained. Using a 1-h pulse with ^{35}S-methionine, thymus certainly contained more labelled NCP150 than spleen or bone marrow (Fig. 1) but unknowns of turnover time and cellular heterogeneity make statements about relative concentrations of NCP150 premature. The detection of NCP150 in spleen and marrow is near the borderline of our present techniques. Using these techniques, cultured fibroblasts from a variety of mouse strains and liver cells have shown no evidence of NCP150. It seems likely from the present results that NCP150 shows a significant variation in concentration as a function of cell type. It also seems from comparisons of band intensities on autoradiograms that the amount of P120 is 40- to 100-fold higher per cell in A-MuLV-transformed cells than the amount of NCP150 in lymphoid tissues. Various cultured murine thymic leukaemia cells have been surveyed and while all showed evidence of NCP150, none was especially high (data not shown).

Work in the avian sarcoma virus system (ASV) has demonstrated the utility of tumour regressor antisera for identifying the normal cellular protein analogue of the transforming gene product, pp60^{src}. In the ASV system the normal cellular pp60 and viral pp60^{src} proteins were very close in molecular weight. The normal cellular pp60 was found in fibroblast tissues of a variety of avian and other vertebrate species but the amount of normal cellular pp60 was 10- to 100-fold less than pp60^{src} in ASV transformed cells[11,12]. The ASV and A-MuLV systems appear similar in that sera that recognise the transforming protein—or the putative transforming protein in the case of A-MuLV—also recognise a normal cellular constituent. The systems differ, however, in that ASV appears to contain a total cellular gene with no added viral determinants while A-MuLV has only part of a cellular gene (about 90,000 of a total MW of 150,000) attached to amino acid sequences of normal M-MuLV proteins. The systems also differ in that the messenger RNA and presumably the protein representing the normal *src* equivalent is present at a low but consistent level in all cells and tissues examined[13] but NCP150 is more variable in occurrence. Both normal cell proteins occur at concentrations much lower than their counterparts in virally transformed cells.

Our results should be contrasted with those of Risser *et al.*[14]. They have prepared a similar mouse anti-A-MuLV syngeneic regressor serum and used it to identify an A-MuLV related tumour cell surface antigen by cytotoxicity. No biochemical determination of the nature of this antigen has been reported. Using lymphoid cells of normal mice in absorption tests, they reported that a cross-reacting antigen can be detected on the surface of bone marrow and spleen cells but not thymus from BALB/c and closely related strains. Unrelated strains, like C57BL/6, were negative for this normal cellular antigen. Our results with NCP150 show a different tissue distribution from that described by Risser *et al.* and we do not find any evidence of allotypy in our serum. Whether the differences between the

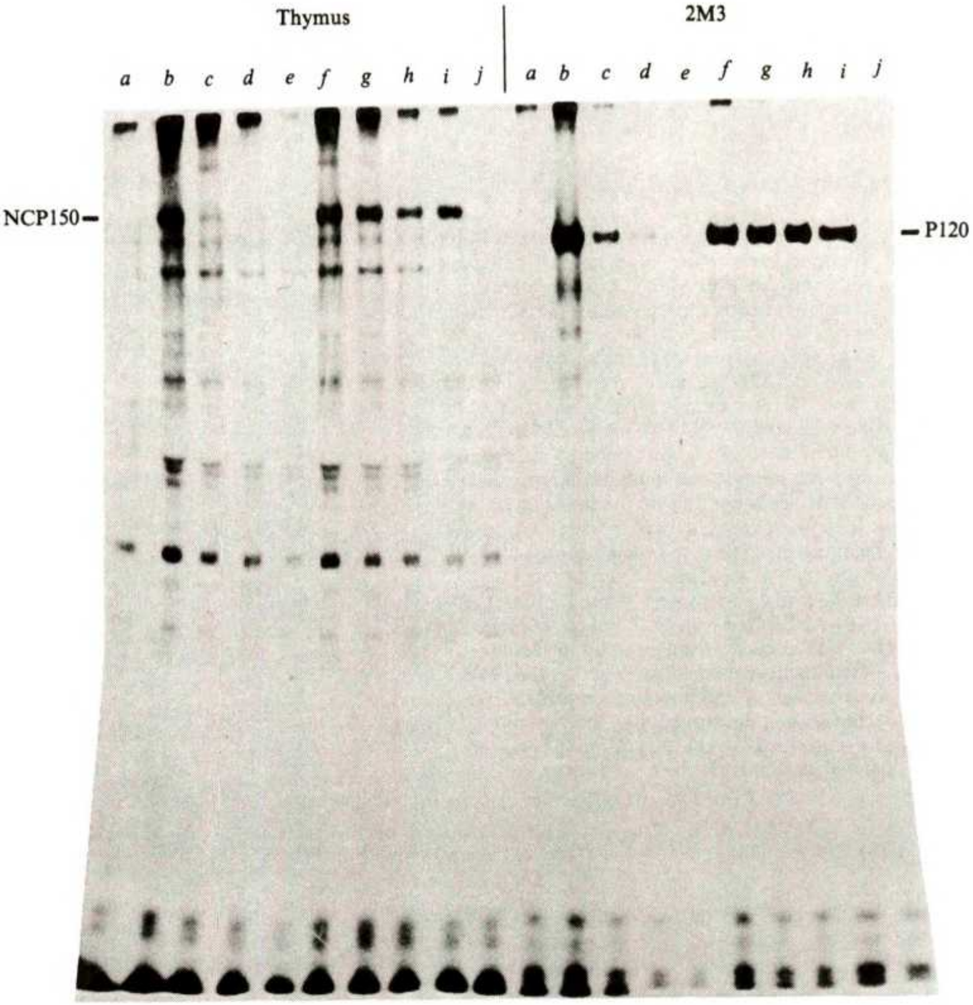

Fig. 2 Competition and absorption analysis of NCP150 and P120. Labelled cell extracts of C57BL/6 thymus and 2M3 cells (an A-MuLV *in vitro* derived clonal lymphoid cell line)[6] were prepared and analysed as in Fig. 1. A final volume of 1 ml for each cell extract of thymus (10^7 cells) or 2M3 (5×10^5 cells) was used for each tube. All tubes contained 100 μg ml^{-1} unlabelled Moloney MuLV virion proteins. Lane *a*, normal mouse serum (5 μl). Lane *b*, anti-AbT (5 μl). Lanes *c*, *d* and *e*, anti-AbT (5 μl) plus unlabelled extract of 2×10^6, 4×10^6 or 8×10^6 A-MuLV transformed non-producer NIH/3T3 cells. Lanes *f*, *g*, *h*, anti-AbT (5 μl) plus unlabelled extract of 2×10^6, 4×10^6, or 8×10^6 Moloney sarcoma virus transformed non-producer NIH 3T3 cells respectively. Lane *i*, 1:10 diluted anti-AbT (50 μl) control-mock absorbed. Lane *j*, 1:10 anti-AbT (50 μl) pre-absorbed with viable A-MuLV transformed non-producer NIH 3T3 cells (2×10^8 cells per ml diluted serum—3 cycles each 45 min; 0 °C). Immune precipitates were prepared and analysed as in Fig. 1.

results imply that we are studying different proteins remains unclear. One possibility is that Risser *et al.* were detecting NCP150 but that the synthetic rate (as monitored by immunoprecipitation) is controlled separately from expression at the cell surface (as monitored by cytotoxicity absorption).

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Clostridium botulinum can grow and form toxin at *p*H values lower than 4.6

G. J. M. Raatjes & J. P. P. M. Smelt

Unilever Research Vlaardingen, PO Box 114, 3130 AC Vlaardingen, The Netherlands

It is generally accepted[1] that in *Clostridium botulinum* both growth and toxin formation are completely inhibited at *p*H values below 4.6. This critical *p*H value has been confirmed by many investigators using food as substrate[2–5] or culture media[3,6–8]. Occasionally[9–12] growth of *C. botulinum* and toxin formation at *p*H values lower than 4.6 have been reported. In these cases the authors ascribed the unexpected outgrowth and toxin formation to local *p*H differences in inhomogeneous media and growth of *C. botulinum* before *p*H equilibration, or to the fact that fungi created microenvironments within or adjacent to the mycelial mat, where the *p*H was higher than 4.6 as was demonstrated by Odlaug and Pflug[11,12]. We show here that the general assumption that *C. botulinum* does not grow below *p*H 4.6 is incorrect. We have observed that growth and toxin formation by *C. botulinum* can take place in homogeneous protein rich substrates (containing 3% or more soya or milk protein) at *p*H values lower than 4.6.

Table 1 Influence of *p*H, type of acid, and protein content on the behaviour of *C. botulinum* type A and B, if inoculated together with *Bacillus* spores in substrates based on protein enriched soya extracts

Acid	Initial *p*H	Protein content (% w/w)		Incubation (weeks at 30 °C)							
				1	2	4	6	8	10	12	14
HCl	4.0	5.5	(1)	+−	−−	−−	++	−+	−+	−−	−+
			(2)	−−	−−	−−	++	−+	−+	−−	−+
			(3)	*θ*	*θ*	*θ*	II	I	I	*θ*	I
HCl	4.2	5.5	(1)	−−	+−	++	−+	−+	−−	++	−+
			(2)	−−	−−	++	−+	−+	−−	−+	−+
			(3)	*θ*	*θ*	II	I	I	*θ*	I	I
HCl	4.4	5.5	(1)	++	++	++					
			(2)	++	++	++			NT		
			(3)	I	II	II					
HCl	4.4	3.0	(1)	−+	−+	−−	−+	−−	−−		
			(2)	−+	−+	−−	−+	−−	−−	NT	
			(3)	I	I	*θ*	I	*θ*	*θ*		
HCl	4.4	0.6	(1)	−−	−−	−−	−+	++	++	−−	++
			(2)	−−	−−	−−	−−	−−	−−	−−	−−
			(3)	*θ*	*θ*	*θ*	*θ*	*θ*	*θ*	*θ*	*θ*
Citric acid	4.4	5.5	(1)	+−	+−	++	++	++	−+	++	++
			(2)	−−	−−	−+	++	++	−+	++	++
			(3)	*θ*	*θ*	I	II	II	I	II	II
Lactic acid	4.4	5.5	(1)	−−	−−	−−	−−	−−	−−	−+	−−
			(2)	−−	−−	−−	−−	−−	−−	−+	−−
			(3)	*θ*	*θ*	*θ*	*θ*	*θ*	*θ*	I	*θ*
Acetic acid	4.4	5.5	(1)	−−	−−	−−	−−	−−	−−	−−	−+
			(2)	−−	−−	−−	−−	−−	−−	−−	−+
			(3)	*θ*	*θ*	*θ*	*θ*	*θ*	*θ*	*θ*	I

(1) Initial inoculum 100 bacilli per ml substrate. At least hundred-fold growth of *Bacillus* in one sample (+) or two samples (++). Less than hundred-fold growth of *Bacillus* in one sample (−) or two samples (−−).

(2) Initial inoculum 1,000 *C. botulinum* per ml substrate. At least 10-fold growth of *C. botulinum* in one sample (+) or two samples (++). Less than 10-fold growth of *C. botulinum* in one sample (−) or two samples (−−).

(3) *θ*, Two samples tested for toxin, no sample toxic; I, 1/2 samples toxic; II, 2/2 toxic.

NT, Not tested.

Media in screwcap bottles containing different levels of soya or milk proteins, in which the *p*H was adjusted with various acids (Table 1), were inoculated with either a mixture of spores of *C. botulinum* type A strain 62A, Vl_1 and ZK_3 and *C. botulinum* type B strain B6 and 2345, alone or together with a mixture of spores of *Bacillus subtilis* and *B. licheniformis* isolated from soya concentrates. Details are given in Table 1.

The inoculated media were filled into screwcap bottles which were subsequently heated for 5 min at 100 °C. After cooling, the headspace in each bottle was filled with liquid paraffin. Duplicate bottles were examined at various intervals during incubation at 30 °C for growth, *p*H value and toxin; the presence of toxin was tested by intraperitoneal injection of mice.

The *p*H value did not increase from the initial values by more than 0.1 *p*H unit during incubation. Growth and toxin formation by *C. botulinum* took place at a *p*H value as low as 4.0 in the presence of the bacilli if the *p*H was adjusted with hydrochloric acid. Growth of the bacilli removed residual oxygen and lowered the redox potential, thus creating more favourable conditions for the growth of *C. botulinum*. When we compared the inhibitory action of hydrochloric, citric, lactic and acetic acids at *p*H 4.4, growth and toxin formation decreased in that order (Table 1).

The level of protein in the media also affected the ability to grow at low *p*H values—at *p*H 4.4 a minimum protein level around 3% was necessary for growth and toxin formation.

In further experiments with 5.5% soya protein J. S. Crowther (personal communication) observed that in anaerobic conditions spores of *C. botulinum* are able to outgrow and form toxin at *p*H 4.2 (if hydrochloric acid was used as acidulant) in the absence of other microorganisms. However the results at this *p*H were not always repeatable.

So far we have not found *C. botulinum* to grow in a wide range of actual foods with *p*H value lower than 4.6, but the assumption that *C. botulinum* does not grow below *p*H 4.6 has been shown to be incorrect, and the understanding of conditions in which growth may occur, requires further study.

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Superoxide involvement in the bactericidal effects of negative air ions on *Staphylococcus albus*

E. W. Kellogg III*, M. G. Yost†, N. Barthakur‡ & A. P. Kreuger‡

* Membrane Bioenergetics Group, Lawrence Berkeley Laboratory and the Department of Physiology-Anatomy, University of California, Berkeley, California 94720
† Department of Biomedical and Environmental Health Sciences, School of Public Health, University of California, Berkeley, California 94720
‡ Department of Agricultural Chemistry and Physics, Macdonald Campus of McGill University, Ste. Anne de Bellevue, Quebec H0A 1C0, Canada

The physical nature of small air ions is well established and it is recognised that they can produce a variety of biological effects[1]. However, in only a few instances have any underlying biochemical changes been detected[2–4]. Theoretically, one can consider the hydrated superoxide radical anion $(O_2^-)(H_2O)_n$ with $n \simeq 4$–8 as a likely candidate for a biologically active species of negative air ion[5]. The chemical and biological reactivity of superoxide is high[6] and includes a leading role in bacterial killing caused by radiation[7,8], in which superoxide dismutase (SOD), an enzyme that catalyses the reaction:

$$O_2^- + O_2^- + 2H \rightarrow H_2O_2 + O_2$$

protected markedly. Other studies have also demonstrated the bactericidal effect of O_2^- (refs 9–11). Inasmuch as the bactericidal action of small negative air ions has been repeatedly confirmed, we decided to test for the involvement of O_2^- in this phenomenon by evaluating the protective effect of SOD. Our results show strong O_2^- involvement in negative air ion bacterial kill.

Figure 1 illustrates the apparatus used in our experiments. To ensure that all negative ions went through the solution to the ground wire, we insulated the ground wire using heat shrink tubing and Teflon tubing to a point well below the solution's surface. A Modulion corona discharge type negative ion generator provided the ionising potential; to maintain a uniform current we adjusted the ionising potential using separate variable transformers on the a.c. lines of each generator.

Inoculated flasks, containing *Staphyloccus albus* at ~10^6 cells per ml in 40 ml of sterile 3.75 mM NaCl with 1.25 mM KPi, *p*H 7.8 buffer, were maintained at 22 ± 1 °C in a waterbath for the duration of the experiment. A vacuum pump with airflow regulator exhausted air from the flasks at a rate of approximately 500 ml per min. Aliquots of 1 ml were removed hourly, serially diluted, and then plated as previously described [12].

We isolated superoxide dismutase from bovine liver using the procedure of McCord and Fridovich[13]. This yielded pure enzyme of high specific activity (~3,500 U ml^{-1}). Catalase (Boehringer Mannheim) was extensively dialysed before use. Enzymes were sterilised by passage through a Millipore filter immediately before addition to the bacterial inoculum.

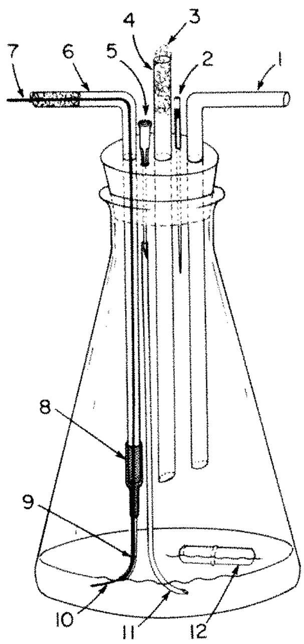

Fig. 1 1, Air exhaust tube. 2, Ion discharge needle. 3, Cotton plug. 4, Air inlet tube. 5, 18 gauge hypodermic needle. 6, Ground wire tube. 7, Ground wire. 8, Heat shrink tubing. 9, 1-mm Teflon insulating tube. 10, Bare platinum tip. 11, 1-mm Teflon sample tube. 12, Magnetic stirring rod. The apparatus was constructed in a 500 ml Erlenmeyer flask fitted with a no. 10 rubber stopper.

Table 1 shows that the exposure of bacteria to negative air ions caused a substantial loss in viability in as short a period as 2 h, with a total loss of viability seen after 5 h of ion treatment. Control samples, incubated in identical conditions but with no negative ionisation, retained full viability after 5 h. Addition of 10 μg ml^{-1} SOD protected bacteria completely against negative ion killing while catalase or denatured SOD had no significant protective effect. The lack of effect of catalase in this system appears in marked contrast to that seen in most systems which involve O_2^- toxicity. For example, both O_2^- and H_2O_2 were required for lipid peroxidation or erythrocyte lysis[14] where their toxicity manifests through their interaction to produce hydroxyl radical and singlet oxygen. In the case of negative air ion bacterial kill the toxicity of O_2^- appears H_2O_2 independent. A possible mechanism might involve O_2^- acting as a nucleophile on the phospholipid bilayer, causing a de-esterification of fatty acids[15]. This could lead to an increase in surface charge and a weakening of the membrane, which under hypotonic conditions might lead to cell lysis and death.

The experiments reported here support the concept that the negative air ions responsible for bacterial death consist solely or in part of hydrated superoxide anions. Paradoxically, while negative air ions have a reputation as a rather beneficient species[1], superoxide radical anion apparently acts as one of the

Table 1 Bacterial kill by negative air ions (% viability)

Conditions	Incubation time (h) 0	1	2	3	4	5
No (−) ions (control)	100	123 ± 3.4	115 ± 22.5	112.5 ± 1.0	111.6 ± 22.3	105.6 ± 11.0
(−) Ions	100	116 ± 3.5	72.3 ± 9.9	29.2 ± 24.9	2.5 ± 2.3	0
(−) Ions +10 μg ml^{-1} SOD	100	129.0 ± 1.0	106.2 ± 18.8	134.0 ± 13.0	109.0 ± 25.1	104.8 ± 14.2
(−) Ions +10 μg ml^{-1} (denatured autoclaved SOD)	100	87.9 ± 7.1	70.0 ± 18.1	27.1 ± 1.1	5.5 ± 1.4	0
(−) Ions +10 μg ml^{-1} catalase	100	105.2 ± 23.8	56.7 ± 10.4	21.5 ± 4.4	11.9 ± 3.4	1.0 ± 1.0

Negative ion flux was maintained at approximately 5 μA in all experiments. ± Figures indicate the range between duplicate experiments.

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most toxic species produced in aerobic metabolism[6]. Perhaps the resolution of this paradox lies in the relative amount of O_2^- required for an effect. Negative air ions can cause physiological effects at extremely low levels, with clean country air having a negative ion concentration of only about 10^3 cm^{-3}, an amount which more closely parallels that of pheromones than of chemical reactants. In our system we have greatly amplified the flux of negative ions to a level which could produce, theoretically at least, 0.3 mmol O_2^- per h, which we channelled through a total of 40 ml of solution. Even so, *in vitro* systems testing for O_2^- reactivity have invoked a flux hundreds of times greater. Thus the effects of superoxide as a negative air ion although dependent on its chemical reactivity may be greatly amplified by a biological sensitivity in the hormonal range. Our tentative identification of $(O_2^-)(H_2O)_n$ as the negative air ions responsible for killing micro-organisms should provide a more solid base for investigating the effects and mechanisms of negative air ion effects in other biological systems.

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Mitochondrial recombination in cytoplasmic hybrids of *Nicotiana tabacum* by protoplast fusion

Geneviève Belliard*, Fernand Vedel† & Georges Pelletier*

* Laboratoire d'Amélioration des Plantes, Bât. 360, Université Paris-Sud 91405, Orsay, France
† Laboratoire de Biologie Moléculaire Végétale associé au CNRS (LA, 40) Bât. 430, Université Paris-Sud, 91405 Orsay, France

We have previously regenerated tobacco plants from fused protoplasts isolated from two varieties of *Nicotiana tabacum*. The parent cells had distinct morphological nuclear and cytoplasmic markers, enabling us to recognise, among the whole regenerated plants, those with a single parental nucleus and a hybrid cytoplasm produced by the mixing of the two parental cytoplasms[1]. Genetic analysis of their progeny has confirmed that the phenotypes of these cytoplasmic hybrids (or cybrids) are stable and maternally inherited. Analysis with restriction endonuclease has shown that only one or the other parental chloroplast DNA is present in the progeny of the cybrids[2]. We report here, however, that the mitochondrial (mt) DNAs of cybrids are different from those of the parents and from the mixture of the two. The new DNAs result from mitochondrial recombination.

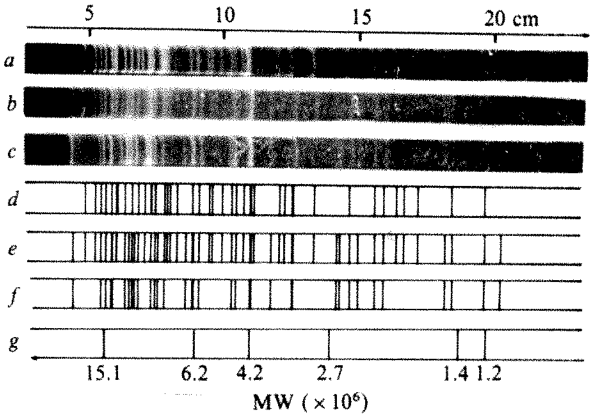

Fig. 1 Agarose slab gel electrophoresis of *Sal*I digests of mtDNAs from: *a*, Ts or *N. tabacum* var. Techne (*debneyi* cytoplasm); *c*, Xf or *N. tabacum* var. Xanthi (*tabacum* cytoplasm); *b*, mixture of Ts and Xf. These diagrams are schematically represented on *d*, (Ts); *e*, (Ts + Xf); *f*, (Xf). *Hind*III fragments of λ DNA (g) were used as standards for molecular weight determinations. Mitochondria were isolated as previously described[5] with the following modifications. Green leaves were used because etiolated material cannot be obtained with tobacco. DNase-treated organelles were purified by centrifugation on two layers of 30 and 60% sucrose. mtDNA was extracted as described for cp-DNA[6,7] and purified by linear CsCl–ethidium bromide gradient centrifugation for 2 d at 32,000 r.p.m. in a SW41 rotor. This gives cleaner mtDNA bands than does the two-step procedure used before. *Sal*I enzyme was prepared according to R. J. Roberts (unpublished). Experimental conditions for mtDNA digestion, agarose gel electrophoresis and UV fluorescence photography were as described[5,7].

Cytoplasmic hybrids were obtained from fusion between protoplasts of *N. tabacum* var. Xanthi (Xf) which has normal fertile flowers and petiolated leaves, and *N. tabacum* var. Techne (Ts), which has abnormal flowers with cytoplasmic male sterility, producing no pollen. The Ts variety, obtained from the interspecific cross *N. debneyi* × *N. tabacum*, has the cytoplasm of the former and the nucleus of the latter. The resulting nucleocytoplasmic interaction is responsible for the male sterility. Ts has sessile leaves, and in our experimental system leaf shape is the nuclear marker and flower shape the cytoplasmic marker. Details of the parents and cybrids are shown in Table 1.

Restriction endonuclease was used to analyse mtDNAs extracted from the progeny of nine cybrids (six Ti and three Xi) which represent all the variability of the new i phenotypes (see legend to Table 2). Mitochondria and mtDNAs were isolated as described in Fig. 1 legend.

Figure 1 shows the *Sal*I restriction patterns for mtDNA from the Ts (*a*) and Xf (*c*) parents showing 38 and 31 fluorescent bands, respectively. Of these, 20 appear to be common to *N. debneyi* and *N. tabacum* mtDNAs, 18 specific to Ts (*N. debneyi*) mtDNA and 11 specific to Xf (*N. tabacum*) mtDNA, as shown by comparison of Ts, Xf and Ts + Xf schematic diagrams (Fig. 1). Consequently, the diagram corresponding to a mixture of the two parent mtDNAs has 49 bands (Table 2). We found that the two varieties of *N. tabacum*, Xf and Tf (fertile Techne containing the *N. tabacum* cytoplasm), had identical mtDNAs. Figures 2 and 3 show the *Sal*I restriction patterns of mtDNAs

Table 1 Genotypes and phenotypes of parent and cybrid plants

Name		Genotypes		Phenotypes	
		Nucleus	Cytoplasm	Leaf	Flower
Parents	Ts	*N. tabacum* var. Techne	*N. debneyi*	Sessile	Abnormal; male sterile type s
	Xf	*N. tabacum* var. Xanthi	*N. tabacum*	Petiolated	Normal; male fertile type f
Cybrids	Ti	*N. tabacum* var. Techne	hybrid *N. debneyi* × *N. tabacum*	Sessile	Intermediate between those of the parents
	Xi	*N. tabacum* var. Xanthi	hybrid *N. debneyi* × *N. tabacum*	Petiolated	

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Table 2 *Sal*I digests of mtDNAs

	Plants	Phenotype	Bands					MW* (×10^6)
			Common	*debneyi*	*tabacum*	New	Total	
	Xf, Tf	Fertile	20	—	11	—	31	206
	Ts, Tsp	Sterile	20	18	—	—	38	256
Ti	T_{161}	I	20	8	7	3	38	293
	T_{47}	I	18	9	7	1	35	269
	T_{27}	I	19	12	7	1	39	264
	T_1	II	17	12	5	4	38	260
	T_{75}	III	17	15	3	2	37	267
	T_{13}	IV	20	18	1	—	39	259
Xi	X_6	II	20	16	3	1	40	248
	X_1	IV	19	17	3	2	41	296
	X_7	IV	20	17	1	1	39	290

Xf, Tf: Varieties with *tabacum* cytoplasm, normal and fertile flowers. Ts: Variety with *debneyi* cytoplasm, abnormal and male sterile flowers with split corolla, short stamen filament ended by a stigmate. Tsp: Control plant: plant regenerated from Ts protoplast with abnormal and male sterile flowers identical to Ts parent ones. Ti and Xi: cybrid plants are defined in Table 1. i Types: I, normal corolla, filament shorter than the normal, anther with some pollen: fertile. II, slightly nicked petals, long filament with flat anther: the most often sterile. III, nicked petals, long filament with distorted anther: sterile. IV, split corolla, short filament ended by white outgrowths: sterile.

* Approximate molecular weights obtained as described in the text.

isolated from the progeny of the regenerated cybrids Ti and Xi, obtained by crossing, respectively, female Ti × male Tf and female Xi × male Xf. Each of the nine cybrids in Table 2 contained a unique mtDNA distinguishable from both parent mtDNAs and from a mixture of the two. However, the cybrid patterns always contained mtDNA fragments belonging to both parents, and also, in all but one, new bands appeared. The number of bands on the cybrid diagrams varied from 35 to 41, confirming that these mtDNAs were not products of the addition of two parental mtDNAs.

The complexity of the restriction patterns precludes accurate determination of molecular weight. Nevertheless, estimates obtained by summing the molecular weights of the bands (band multiplicities are not taken into account and it is difficult to determine precisely the size of the larger fragments) show that the cybrid mtDNAs more closely resemble the Ts mtDNA than the Xf mtDNA or the mixture of parental mtDNAs (Table 2). Table 2 also shows for each cybrid mtDNA the distribution of the specific and common parental bands. There is clearly a correlation between flower shape and male sterility or fertility and the ratio of the specific *N. debneyi* and *N. tabacum* bands: an increase in the degree of sterility in the different i phenotypes of the cybrids corresponds to an increase in the number of *N. debneyi* specific fragments.

The fate of mtDNA after the initial fusion of *N. debneyi* and *N. tabacum* cytoplasms is strikingly different from that of chloroplast DNA in our cybrid plants[2]. Our results, together with previous observations of maternal inheritance and stability of floral markers, constitute evidence that new cytoplasms characterised by specific mtDNAs arise from protoplast fusion. Consequently, flower morphology and male sterility in tobacco must be controlled by nucleo–mitochondrial interaction. The presence of new bands in the cybrid mtDNA restriction patterns is evidence of mitochondrial recombination in somatic hybrid cells: the simultaneous presence, in the same cell, of *N. tabacum* and *N. debneyi* mitochondrial genomes is followed by mtDNA recombination because new molecular species are observed. Fonty *et al.*[3] have used restriction enzymes to analyse the mitochondrial genome of yeast cells and have provided the first evidence for physical recombination of mtDNA. They have also indicated that this event is frequent in crosses of wild type yeast cells.

The advantages of somatic cell hybridisation for studying non-chromosomal inheritance in higher plants were first pointed out by Gleba[4]. Unfortunately our knowledge of the mitochondrial genetics of higher plants is still limited by the scarcity of biochemical and genetic markers (namely resistance markers). The cytoplasmic parasexual hybrids of *N. tabacum* analysed here are identified by their floral phenotypes and also by specific mtDNAs arising from recombinations between different mtDNAs. The cytoplasmic male sterility in tobacco involves the mitochondria and can be transferred by protoplast fusion from one variety to another. These results emphasise the potential of somatic cell fusion for studying mitochondrial genetics and for increasing the cytoplasmic variability in higher plants.

We thank Dr F. Quetier for *Sal*I endonuclease and helpful discussions.

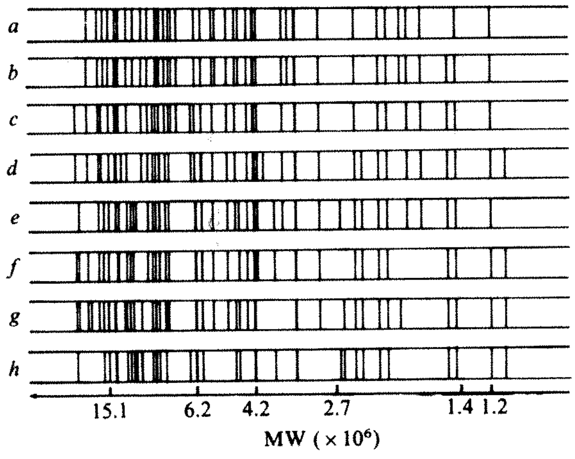

Fig. 2 *Sal*I band patterns on 0.7% agarose gel shown by the mtDNAs from parents: *a*, Ts; *h*, Xf, and from some Ti cybrids of first generation: *b*, T_{13}; *c*, T_{75}; *d*, T_1; *e*, T_{27}; *f*, T_{47}; *g*, T_{161}. Ti plants were characterised by the nuclear genome of the Ts parent (Ti plants and Ts parent presented identical leaf shape) and by hybrid cytoplasms (the flower shapes of the Ti plants were intermediate between the two parental ones). Isolation and analysis of the mtDNAs were as described in Fig. 1.

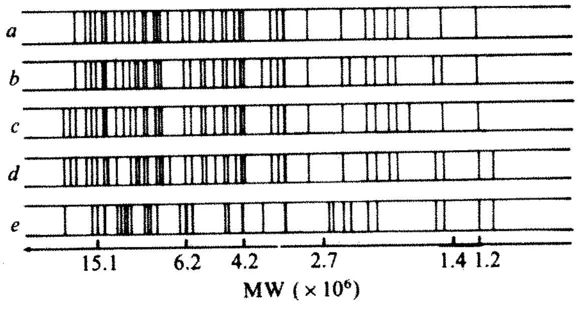

Fig. 3 *Sal*I band patterns on 0.7% agarose gel shown by the mtDNAs from parents: *a*, Ts; *e*, Xf, and from some Xi cybrids of first generation: *b*, X_6; *c*, X_7; *d*, X_1. Xi plants were characterised by the nuclear genome of the Xf parent (Xi plants and Xf parent presented identical leaf shape) and by hybrid cytoplasms (the flower shapes of the Xi plants were intermediate between the two parental ones). Experimental procedure as for Fig. 1.

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Pretreatment with acetylaminofluorene enhances the repair of O^6-methylguanine in DNA

Julie D. Buckley, P. J. O'Connor & A. W. Craig

Paterson Laboratories, Christie Hospital and Holt Radium Institute, Manchester, UK

Epidemiological and experimental evidence suggests that the interplay of environmental factors may be particularly relevant to the induction of cancer in man[1,2]. We have investigated the possible interrelationships, at the level of DNA repair, of two chemically different hepatocarcinogens administered to rats. Animals were pretreated for several weeks by inclusion of 2-acetylaminofluorene (AAF) in the diet and a study was made of the capacity of liver to repair lesions subsequently introduced into DNA by a pulse of dimethylnitrosamine (DMN). Of particular interest was the repair of adducts at the O^6 position of guanine as this product has been implicated as a critical reaction site for carcinogenesis by the monofunctional alkylating agents[3,4]. We show here that the capacity of liver to repair O^6-methylguanine in DNA is enhanced by prolonged exposure of rats to the chemically unrelated agent, AAF.

Male Wistar rats, 7–8 weeks old and weighing ~200 g, were given (*ad libitum*) standard laboratory diet containing 0.06% AAF for three weeks and were then transferred to normal diet for the fourth week. Three such feeding cycles were administered and DMN was given at the end of the final week (Fig. 1). At this stage the animals were 19–20 weeks old and weighed ~270 g, much lower than the weight (400 g) of a normal 20-week-old colony-bred rat. This weight difference was apparently due to the lower food consumption during exposure to the carcinogen diet, as a group of animals kept throughout the 12 weeks on a schedule of equivalent daily food intake per rat also reached a body weight of about 270 g. All animals, AAF-pretreated and controls, received a single intraperitoneal (i.p.) injection of (^{14}C-methyl)-labelled DMN and were later killed at various intervals. Liver DNA was isolated and analysed as described (see Table 1 legend) to determine the amounts of O^6-methylguanine and 7-methylguanine as a proportion of the parent nucleotides. As evidence suggests that 7-methylguanine is lost in a constant and predictable manner from the liver DNA of normal rats by chemical depurination reactions[5] the amounts of O^6-methylguanine were expressed as a ratio of the 7-methylguanine present in the same DNA to provide an estimate of the relative persistence of this O-methylated product. This ratio also provides a convenient internal standard to allow for any effects of toxicity that may lead to cell lysis, which would affect the loss of all DNA products in the same way.

Following DMN treatment at 5.5 mg kg^{-1}, the amounts of O^6-methylguanine and 7-methylguanine present in liver DNA of the control rats kept on an equivalent daily food intake were determined (Table 1). The results are similar to those obtained for weight-matched controls and lie within the expected range[6] both with regard to the amount of base formed and the ratio of O^6-methyl- to 7-methylguanine (compare with Figs 2 and 3). Data from these weight-matched controls have been used for comparison with AAF-pretreated animals. Results presented here are for AAF-pretreated rats with livers of normal appearance. This included the majority of animals (see ref. 7) and the remainder, which had sparsely nodular livers, are included in a separate study. Measurements of the exhalation of radioactively labelled CO_2 (ref. 8) indicated that the rate of metabolism of (^{14}C-methyl)-labelled DMN was similar in both control and AAF-pretreated rats. In agreement with previous reports for normal rats[8] the lower doses were metabolised more quickly but the process was nevertheless complete by 5 h in all cases. At this time the level of 7-methylguanine was similar in liver DNA from control and AAF-pretreated rats (Fig. 2*b*) and in both cases the amount formed was linearly related to dose. The amounts of O^6-methylguanine at 5 h were also dose-related but they were significantly lower for the AAF-pretreated rats, indicating that at all dosages the repair of this product was already well advanced (Fig. 2*a*): A comparison of the relative persistence of this base over a 48 h period further emphasised the differences observed between the two treatment conditions (Fig. 3). Thus, in control animals 5 h after DMN treatment at doses of 5.5 or 9 mg kg^{-1} this ratio of O^6-methyl- to 7-methylguanine approximates to the expected value of 0.115 for DMN[6]. At the highest dose there was a slight increase in this ratio over the next 20 h or so (Fig. 3*a*) and this rise can be accounted for by calculation of the expected loss of 7-methylguanine by chemical depurination, indicating that the amount of O^6-methylguanine had remained constant for this period. After this, the ratio decreases rapidly so that by the 48 h the value approaches that obtained for the lower doses at later times. This inhibitory effect is much less apparent at 5.5 mg kg^{-1} and was not detected at the 1 mg kg^{-1} dose. Also, at 1 mg kg^{-1} the ratio of methylated guanines is much lower, indicating that a greater relative proportion of the O^6-methylguanine initially present in DNA (see Figs 2*a* and 3*a*) was repaired than at the high doses. A similar effect is seen in AAF-pretreated rats but at each dose level the repair of O^6-methylguanine is enhanced, and at 9 mg kg^{-1} this

Table 1 Amounts of O^6-methylguanine and 7-methylguanine in liver DNA of control rats

DNA reaction product	Time after DMN treatment 5 h	 8 h	 24 h
7-Methylguanine*	412	378	275
O^6-Methylguanine*	39.8	36.9	14.4
O^6-Methyl/7-Methylguanine	0.097	0.098	0.053

Control rats, having received daily food intake equivalent to that of the AAF-pretreated animals, (see text) were treated with a single (i.p.) injection of DMN (5.5 mg kg^{-1}, 5 mCi mmol^{-1}). The livers of 3 rats were pooled for each time point, DNA was extracted by a modified phenol procedure[18] and hydrolysed in 0.1 M HCl (70 °C, 30 min). The authentic marker compounds 7-methylguanine, 3-methyladenine and O^6-methylguanine were added and the pH of the hydrolysate adjusted to 2.8. Purine bases were separated by chromatography[19] on columns of (1 × 100 cm) Sephadex G-10 eluted with 0.05 M ammonium formate—0.2% (w/w) sodium azide, pH 6.75 at a flow rate of 15–20 ml h^{-1}. The absorption at 260 nm of each fraction (4.5 ml) was determined and hence the amounts of adenine and guanine were calculated. The samples were dried and counted for radioactivity after addition of water (1.3 ml) and Triton-toluene phosphor (10 ml). These measurements were used to estimate the amounts of methylated purines.

* Amounts of alkylation products are given as μmol methyl per mol DNAP.

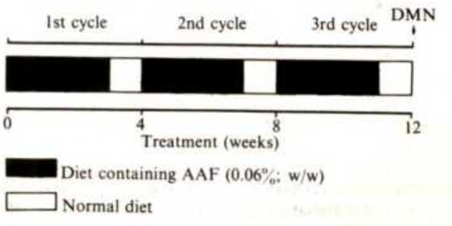

Fig. 1 Schematic representation of dietary regimen.

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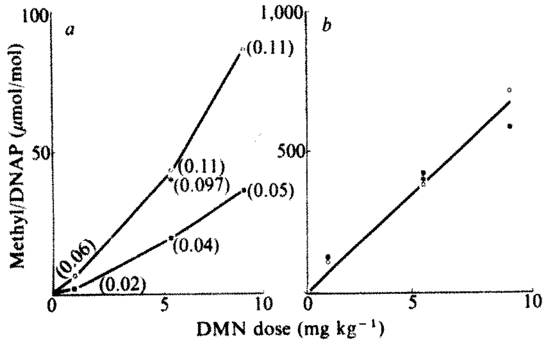

Fig. 2 Amounts of O^6-methylguanine (*a*) and 7-methylguanine (*b*) in the liver DNA of control (○) and AAF-pretreated (■) rats 5 h after administration of (^{14}C-methyl)-labelled DMN at 1, 5.5 and 9 mg kg^{-1} (specific activity 11 mCi mmol^{-1} for lowest dose and 5 mCi mmol^{-1} for higher two doses). Liver DNA was prepared and analysed as previously described (see Table 1 legend) and the 5 h values are taken from lines of best fit for results obtained for animals killed over a 48 h period. Lines of best fit were derived as follows: *a*, quadratic curves ($P < 0.05$ in each case) for AAF-pretreated rats; Gompertz curves were fitted for the control data; *b*, least square regression lines (coefficient of multiple correlation $R > 0.98$ in each case). The ratios of the amounts of O^6-methylguanine to 7-methylguanine are given in parentheses adjacent to the appropriate points. Values of these methyl purines taken from Table 1.

occurs to such an extent that there is no detectable inhibition of the repair process at all (Fig. 3*b*). Some recent reports have shown that chronic treatment with agents which produce O^6-methylguanine in DNA can also induce the repair of these lesions. This has been observed for rat liver DNA following chronic administration of DMN[9], in several organs of the rat following repeated injections of dimethylphenyltriazene[10] and for *Escherichia coli* following pretreatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine[11].

In the AAF-pretreated animals the reason for the observed increase in the ratio of methylpurines at around 8 h (Fig. 3*b*) is obscure, but as metabolism of DMN is complete by the fifth hour no further increase in the amount of alkylation at the O^6-position of guanine could occur. Further, as reincorporation of this product is extremely unlikely[12], one possible explanation might be the induction of a specifically enhanced loss of 7-methylguanine over this short period: such a process has not yet been observed for rat liver DNA but this could be a side effect of the stimulation of the system for the repair of O^6-methylguanine in AAF-pretreated rats. For comparison, evidence for the repair of 7-methylguanine has been given in certain cases, for example in the DNA of the livers of mice[13] and of Syrian hamsters[14].

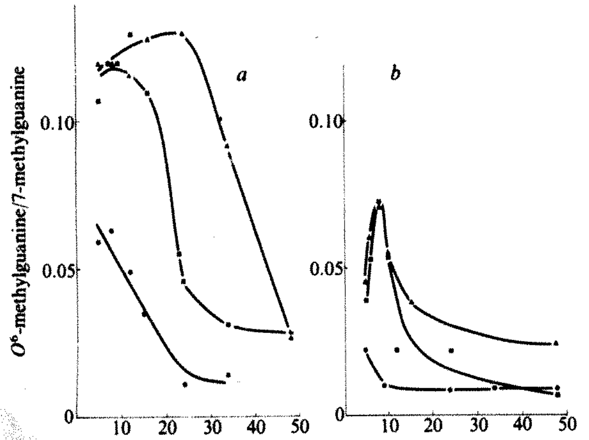

Fig. 3 Ratio of O^6-methyl- to 7-methylguanine in liver DNA in control (*a*) and AAF-penetrated (*b*) rats at various times after injection (i.p.) of (^{14}C-methyl)-labelled DMN at 9 mg kg^{-1} (▲), 5.5 mg kg^{-1} (■) and 1 mg kg^{-1} (●).

In conclusion, we have observed a dose-related repair of O^6-methylguanine in normal rats which, in agreement with previous work[6,15], is relatively more active at lower doses of DMN. Furthermore, a similar repair process is also present in AAF-pretreated rats, but here the repair of O^6-methylguanine is significantly enhanced at all doses used (Fig. 3). This effect is particularly evident at early times and when compared to controls there is a two- to threefold decrease in the ratio of these methylated guanines. It is interesting that a prolonged exposure to one carcinogen can induce the repair of lesions formed in DNA by another chemically unrelated carcinogen. Such an effect might be due to the induction of a general repair mechanism, possibly analogous to the induction of post-replication repair observed in AAF-treated Chinese hamster cells[16]. Alternatively, this might also be explained by the activation of a more specific repair system due to the formation of small amounts of an AAF-adduct at the O^6-position of guanine in DNA, particularly as such lesions have now been reported for the reaction of another aromatic amine, 1-naphthylamine, with DNA[17]. The formation of a lesion at a common site and its subsequent control by similar or closely related repair systems, which is implicit in the latter interpretation, may be especially relevant to the concept of mechanisms of carcinogenesis arising from agents belonging to different classes of chemical carcinogens.

We thank ICI Pharmaceuticals Division for the gift of the acetylaminofluorene diet and Dr M. K. Palmer and Mr R. Swindell for statistical treatment of the data. This work was supported by grants to the Paterson Laboratories from the MRC and the Cancer Research Campaign.

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Corrigendum

In the letter 'Use of Panamanian sea urchins to test the molecular clock' by H. A. Lessios, *Nature* **280**, 599–601, line 4 in paragraph 6 should read '. . . in *Diameda* were 20 times higher than in *Echinometra*, we would . . .'

Erratum

In the letter 'Induction of sporulation in *Bacillus brevis* by peptide antibiotics' by H. Ristow *et al.*, *Nature* **280**, 165–166, in paragraph 2 line 6, for ". . . that of gramicidin D between t_1 and t_5.", read ". . . that of gramicidin D between t_1 and $t_{1.5}$." Also for the abscissa on the top of Fig. 1 "$t_1 \ldots t_7$", read "$t_0, t_1 \ldots t_6$".

matters arising

The cosmological evolution of QSOs

RECENTLY, Stewart and Hawkins[1] claimed that what is usually considered strong evidence for cosmological evolution of the number density of quasi-stellar objects (QSOs) is merely a statistical sampling effect. They find that the assumed evolution law, combined with the optical luminosity function of QSOs, predicts results that conflict with the observational data from which these two functions are derived. However, their result is due to several incorrect assumptions, and I suggest there is no such conflict for the largest homogeneous QSO sample available. Cosmological redshifts are assumed here throughout.

The proposal that the observed upper envelope of the points in the redshift–absolute optical luminosity plane is the result of statistical sampling[1] is not new. Schmidt[2] introduced the V/V_m test partly to remove this obvious selection effect, and a related method that also avoids the problem was proposed by Lynden-Bell[3]. In the V/V_m test, the number density evolution law is derived such that the selection effect is taken into account, and the luminosity function is then derived for a particular epoch (redshift) by applying this density law to the observed number densities at other redshifts represented in the sample. Note that the luminosity function cannot be derived independently of the assumed density evolution law. Stewart and Hawkins[1] claim that the upper envelope of the points in the log F_{2500}, log z plane is satisfactorily reproduced only if there is no significant density evolution, and that if the previously-derived evolution law and luminosity function are assumed there is a serious conflict between the predicted and observed envelopes (F_{2500} is the absolute luminosity, in W Hz^{-1}, at 2,500 Å rest wavelength, using a Hubble constant of 100 km s^{-1} Mpc^{-1}, and is related to Stewart and Hawkins absolute magnitude M_v by $\log F_{2500} = 13.32 - 0.4M_v$; z is the redshift). Apart from doubts concerning the use of the upper envelope as an efficient statistical indicator, I believe their calculations to be invalid for the following reasons: (1) if there is no density evolution, it is incorrect to adopt a luminosity function that was derived *via* the assumption of strong evolution; (2) if there is evolution, it is unjustifiable to extend the optical luminosity function to arbitrarily high values when there is clear evidence[3–6] for an upper limit; and (3) Stewart and Hawkins[1] have not taken radio selection effects into account properly. The probability that a radio-emitting QSO will find its way into a catalogue depends on its radio flux density, that is on its absolute radio luminosity and its redshift. This modifies their equation (8) for the number of QSOs detected as a function of z, by an amount that depends on the radio flux density limit. Those QSOs in Stewart and Hawkins[1] inhomogeneous sample (derived from ref. 7) that were originally found as radio sources come from surveys with quite different radio limits, which makes the modification of their equation (8) indeterminate.

Stewart and Hawkins'[1] result therefore seems to be based on erroneous assumptions, and as a check I have calculated the expected distribution of points in the log F_{2500}, log z plane for the largest homogeneous QSO sample published so far[5]. This contains 74 QSOs identified as 4C radio sources, with stringent radio and optical flux density limits. Redshifts, and therefore values of F_{2500}, are known for 71 of them[8]. It has been shown[5] that the luminosity function for the objects is adequately represented by:

$$dN \propto F_{500}^{-2.65} F_{2500}^{-1.08} V^{1.55} \times dF_{500} dF_{2500}\, dV \qquad (1)$$

where F_{500} is the absolute radio luminosity[2] and $V(z)$ is the co-moving volume out to redshift z, using a deceleration parameter $q_0 = 1$. Over the redshift interval represented in the sample, the $V^{1.55}$ density evolution law is virtually indistinguishable from that used by Schmidt[9] and by Stewart and Hawkins[1]. It was shown[3–6] that the optical luminosity function steepens significantly near 10^{24} W Hz^{-1} (Stewart and Hawkins[1] assume that the function can be extrapolated to luminosities ~100 times this value). Using equation (1) with a cutoff at 10^{24} W Hz^{-1}, it is straightforward to derive the expected distribution of points in the log F_{2500}, log z plane (Fig. 1). The agreement is clearly satisfactory, except in the highest luminosity interval which probably indicates that the effective cutoff is somewhere in the interval log $F_{2500} = 23.6$–24.0 (ref. 5). Any remaining inconsistencies in Fig. 1 are trivial in the present context; they probably arise through neglect of possible interdependences of the functions in equation (1).

The agreement between the predictions and observations, shown in Fig. 1, should not be surprising, because the density evolution law and luminosity function used in the calculations were derived from the observations, but it entirely removes the basis for Stewart and Hawkins' dismissal of the substantial evidence for number density evolution derived from

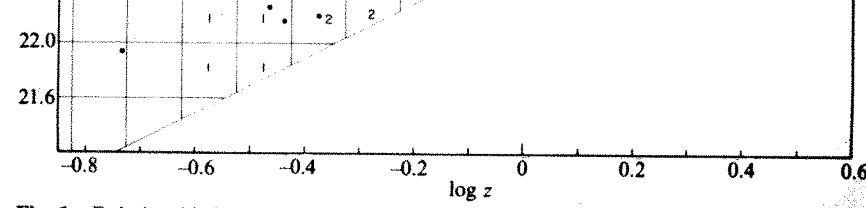

Fig. 1 Relationship between absolute optical luminosity (F_{2500}) and redshift (z) for QSOs in the homogeneous sample (from ref. 5). The predicted distribution is shown as the numbers of QSOs in cells 0.4 wide in logF_{2500} and 0.1 wide in log z, or in the smaller areas if the cell is crossed by the sloping line, which is the limiting apparent magnitude of the sample. The predictions assume an upper limit of 10^{24} W Hz^{-1} in the luminosity function[3–6]. Where no predicted number is given, it is <0.5 per cell. The total numbers of QSOs predicted in the intervals log $z = -0.625$ to -0.725, -0.725 to -0.825, and < -0.825 are 2.0, 1.1 and 1.1 respectively. The points show the observed distribution.

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carefully-compiled homogeneous samples of QSOs.

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D. WILLS

Department of Astronomy,
University of Texas,
Austin, Texas 78712

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STEWART AND HAWKINS REPLY—it seems clear that there is no essential disagreement between Wills' response and the primary result of our original paper[1]; that if one takes the evolutionary law and luminosity function for quasars widely found in the literature and models the (L, z) plot one finds a disagreement with the observations, whether one uses the large list of Burbidge *et al.*[2] or the complete sample of Wills and Lynds[3], in the sense that there is a shortage of very luminous objects. The question now arises as to why this disagreement with the observations exists. Wills accepts that the evolution law and luminosity function are correct and solves the problem by involving a 'cutoff' in the luminosity of quasars, that is by saying that that for some reason a quasar cannot be more luminous than 10^{24} W Hz^{-1} at 2,500 Å.

An attempt was made[1] to discuss the distribution in the (L, z) plot without invoking a cutoff by relaxing some of the assumptions, in particular by allowing the evolution rate to be an adjustable parameter and maintaining the luminosity function unchanged, although allowing rather large error bars. Wills is not strictly correct in saying that the two cannot be distinguished as it is clear[1] that the slope of the upper envelope in the (L, z) diagram at low redshift is determined by the index of the luminosity function alone. The result[1] was that the distribution of quasars in (L, z) could be accounted for very naturally with low or zero evolution, and in particular the absence of very luminous objects was shown to be a simple geometrical effect, thus removing the need for a cutoff in quasar luminosity.

It is obvious that if one regards the results of previous measurements of quasar evolution as being beyond reproach one is forced to introduce a luminosity cutoff and all Wills' results follow. However, as this widely held position is based on the results of a single test—the V/V_m test—it seems to us that one ought to look more closely at this and ask if it is really giving the right result. This question is treated more fully in another paper[4].

If one insists that the V/V_m test is giving the right answer then Wills is quite right and a cutoff, evolution and so on are all required. However, given the results of ref. 1 it seems desirable to re-examine the V/V_m test, as it may be possible to abandon two *ad hoc* hypotheses, that is, quasar evolution and luminosity cutoff, if its results turn out to be incorrect.

N. J. STEWART

Department of Astronomy,
University of Edinburgh, UK

M. R. S. HAWKINS

Royal Observatory,
Blackford Hill,
Blackford, UK

1. Stewart, N. J. & Hawkins, M. R. S. *Nature* **276**, 163–165 (1978).
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Mixed ligand chelate therapy for plutonium and cadmium poisoning

I HAVE accumulated information leading me to doubt the veracity of results published in a report[1] and a reply[2] of which I was the co-author. I have repeated the experiments personally, and thus far find that in the stated conditions[1] I do not confirm these results. The experiments reported for cadmium poisoned mice, for example, did not, contrary to data provided me, include experimental controls for the individual chelants at the stated doses. Further, treatment was given 5 min post-Cd and not 1 h, as stated. At the latter time no survival is observed. Neither can I confirm the results described for plutonium decorporation by diethylenetriaminepentracetic acid/salicyclic acid (DTPA/SA). In fact, from potentiometric titration studies which I now carry out as a screening method for the identification of mixed ligand chelate (MLC) systems potentially useful for biological applications, I find that the DTPA/SA systems do not form significant levels of MLCs with a cation similar to Pu(IV), namely Th(IV). However, DTPA and catechol or Tiron do form MLCs but that with EDTA instead of DTPA is superior.

I believe that MLC phenomena constitute a potentially superior approach for the treatment of metal poisons. However, the extent to which this is realised will be determined by the results of my own experiments and those of others. In the meantime, I am forced to conclude that the previously published results[1] are invalid.

JACK SCHUBERT

Hope College,
Holland, Michigan 49423

1. Schubert, J. & Derr, S. K. *Nature* **275**, 311–313 (1978).
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Mixed ligand chelation therapy

SCHUBERT AND DERR[1] have reported that mixed ligand chelates (MLCs) of DTPA and salicylic acid (SA) markedly enhance the clearance of ^{239}Pu from mice. We have examined this procedure in rats (196–224-d-old females, HMT strain, from the MRC Radiobiology Unit, Harwell) using similar injection procedures and similar analytical procedures. Analysis for ^{239}Pu in excreta from animals caged singly, six animals per group, as well as the kidneys, livers, spleens and carcasses, showed that there was no significant difference between either the DTPA group or the DTPA/SA group.

We have also investigated the extraction of ^{239}Pu into heptane and found no evidence that Pu(EDTA)SA is lipophilic. Unfortunately the extraction procedure was not described; however, our extraction procedure has been used routinely to demonstrate the interaction of ^{239}Pu^{4+} with phospholipids[2].

Furthermore, we have found that intravenously injected NaSA (2.0 mmol per kg) is acutely lethal to rats and it is known that 10–30 g of NaSA is lethal to man[3]; the injection procedure of Schubert and Derr would necessitate intravenous administration of 22 g of NaSA to man every 3 d.

After completing our work we learnt that Schubert had withdrawn some of his claims, but we still view his optimism for MLC therapy with caution. For MLC therapy to be successful it will require administration of a chelating agent sufficiently lipophilic to offset the hydrophilicity of the Pu DTPA complex. There is no reason to believe that such diverse species enter cells in synchrony; in addition, we might wonder if serum citrate could not be part of an MLC with Pu and DTPA. It is more likely that synergistic chelation therapy[4] will contribute more to toxic metal decorporation than MLC therapy.

R. A. BULMAN
F. E. H. CRAWLEY
D. A. GEDEN

Department of Biology,
National Radiological Protection Board,
Harwell, Didcot, Oxon, UK

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Lowland Maya radiocarbon dates and the classic Maya collapse

SIDRYS AND BERGER[1] assert that "the demise of the stela cult and the breakdown of elite culture seem to have coincided" among the Late Classic Maya population of the Southern Lowlands. Their evidence leans heavily on a comparison of frequency distribution graphs for long count-dated Maya monuments and radiocarbon-dated organic materials. The authors claim that the graphs are "remarkably similar", and that their resemblance "helps to establish the reliability of the ^{14}C frequency-distribution bar-graph method".

We feel that the radiocarbon data presented by Sidrys and Berger show such a bias in sampling that the conclusions they draw are unreliable and misleading. Of the 96 radiocarbon dates plotted for the 'elite contexts' (in contrast to 415 monument dates) 66 dates are from the site of Tikal—no other single site is represented by more than 5 dates. Since the authors are attempting to analyse a demographic phenomenon that took place over thousands of square kilometres and involved many population centres, we suggest that selecting nearly 70% of the samples from one site dangerously disregards basic tenets of sampling theory. To demonstrate the effect that such an extreme bias in the radiocarbon data can have on the interpretation made by the authors, we have replotted their frequency-distribution graph for elite contexts, specifically excluding the dates from Tikal. The dates from all of the other 26 sites and artefacts that are listed in their Table 1 have been left unchanged. For comparison we have also reproduced the authors' original graph, which includes the Tikal data.

The replotted data (Fig. 1*b*) fail to support any significant 'breakdown of elite culture' during the eighth to eleventh centuries AD It is not our intention to question whether or not a breakdown actually took place. Our point is that such a drastic difference in interpretation, based solely on the presence or absence of data from a single site, renders spurious Sidrys and Berger's argument that a connection between the demise of the stela cult and the breakdown of elite culture is supported by a similarity between the frequency distribution of monument dates and elite context radiocarbon dates.

While we endorse the trend towards increasing quantification of archaeological data, particularly for the purpose of hypothesis testing, we emphasise that studies involving demography must take into account the need for representative sampling of available sites and contexts.

I. STERNS
C. LORIA
Department of Anthropology,
Cornell University,
Ithaca, New York 14853

R. GARBER
Section of Botany, Genetics and Development,
Cornell University,
Ithaca, New York 14853

1. Sidrys, R. & Berger, R. *Nature* **277**, 269–274 (1979).

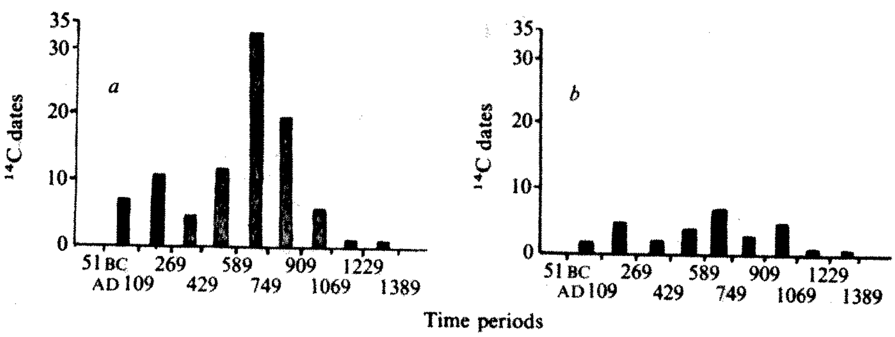

Fig. 1 *a*, Radiocarbon dates from elite contexts in the Southern Lowlands per time period, including dates from Tikal. *b*, The same data as in *a*, but excluding the dates from Tikal.

SIDRYS AND BERGER REPLY—Our study did take into account the need for representative sampling of available sites. The high proportion of Tikal cases in our ^{14}C graph of elite contexts simply reflects the abnormally large number of aristocrats, priests and administrators present at Tikal—a factor that Sterns, Loria and Garber overlook. Population estimates for Classic Maya centres are difficult to obtain and their reliability may be off by at least a factor of two. Nonetheless, those studying the Mayan culture are in general agreement that Classic period Tikal was the largest population centre, with about 50,000 inhabitants[1] (some estimates are as high as 80,000). The number of Tikal elite has been estimated[2] to be 1,000. In contrast the average population of the other 18 sites used in our Fig. 3*a*, according to our own 'guestimates' based on house mounds and size of ceremonial area, as well as the little published demographic data available, falls somewhere between 3,000 and 9,000.

Our sampling procedure thus mirrors Classic Maya cultural reality—the overwhelming economic and political dominance of the capital city of Tikal. Viewed from this perspective, the above graph by Sterns, Loria, and Garber that eliminates Tikal from the total elite sample is the equivalent of a demographic study of the state of New York that does not include New York City. At the same time, we do emphatically reiterate that more Maya ^{14}C dates from commoner contexts are urgently needed to resolve the issue of the Classic Maya collapse.

RAYMOND SIDRYS
RAINER BERGER

Department of Anthropology,
Geography and Institute of Geophysics,
UCLA, California 90024

1. Haviland, W. *World Archaeol.* **2**, 186–197 (1970).
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Matters Arising

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Ca^{2+} and photoreceptor adaptation

FLAMING AND BROWN[1] seem to have missed the point of our earlier work on the effects of altered extracellular Ca^{2+} on rod photoreceptor responses[2], and they give a misleading impression of the possible role

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of Ca^{2+} in mediating sensitivity changes that occur in rods during light and dark adaptation. We found that altering extracellular Ca^{2+} alters the amplitude of rod responses dramatically in the dark-adapted retina, but that the sensitivity of the rod (determined principally by σ, the position of the V–log I curve on the intensity axis) is virtually unchanged. As both amplitude and sensitivity vary substantially during light and dark adaptation, we concluded that changes in cytosol Ca^{2+} levels could not explain adaptation in photoreceptors. For example, we showed (Fig. 5 of ref. 2) that raising extracellular Ca^{2+} from 1.8 to 3.2 mM reduced V_{max} of the rod response by about 38%, but that in these conditions of high extracellular Ca^{2+} a significant shift in the position of the V–log I curve on the intensity axis could not be detected. There is, of course, some uncertainty in intracellular recording measurements, and therefore it is possible that within our error range (±0.2 log units) some small changes did occur. However, such changes are insignificant compared to the changes that occur when a rod is adapted by a light that reduces its maximum amplitude response by the same amount that 3.2 mM Ca^{2+} reduces V_{max}. For example, Fig. 6 of ref. 2 showed that an adapting light which reduced the rod response amplitude by 35% caused a shift of the V–log I curve along the intensity axis by at least 1.6 log units. In other words, in comparable conditions of V_{max} reduction, light causes a 65-fold change in rod sensitivity, but raised $[Ca^{2+}]_o$ changes sensitivity no more than 2–3 times. This was the main point on which our conclusion was based.

Flaming and Brown now report that if they raise or lower extracellular Ca^{2+} they can detect small changes in the position of the rod V–log I curve on the intensity axis and they suggest, therefore, that it is possible that alterations in cytosol Ca^{2+} levels can account for sensitivity changes in photoreceptors during light and dark adaptation. They report that raising extracellular Ca^{2+} from 1.8 to 6 mM causes the V–log I curve of the dark-adapted rod to shift by an average of 0.26 log units. In their Fig. 1 (ref. 1), they show that such an increase in $[Ca^{2+}]_o$ decreases a rod V_{max} by about 45%. If light is used to reduce V_{max} by this amount, however, the position of the V–log I curve alters not by 0.2–0.3 log units, but by about 2 log units. Thus, their data are in close agreement with our findings, and they support our conclusion that, "although cytosol Ca^{2+} concentrations are capable of regulating the membrane potential of the cell and its response amplitude, they affect only to a minor extent the sensitivity of the receptor cell in the dark-adapted or partially light-adapted state" (ref. 2).

Bastian and Fain[3] have recently carried out experiments similar to those of Flaming and Brown and ourselves. They found, in agreement with Flaming and Brown, that small shifts in the V–log I curve can be detected when extracellular Ca^{2+} is altered sufficiently, but their results also show clearly that the effects of increases in extracellular Ca^{2+} do not at all resemble the effects of background light on either rod sensitivity or response waveform. We may not have observed the small shifts of the V–log I curve in low and high Ca^{2+} because of the reasons outlined by Flaming and Brown. However, our failure to observe these changes in no way alters our conclusion that cytosol Ca^{2+} levels cannot be the major determinant of rod sensitivity during light and dark adaptation. Indeed, all three studies discussed here provide evidence in favour of this conclusion.

STUART A. LIPTON
SANFORD E. OSTROY
JOHN E. DOWLING

*The Biological Laboratories,
Harvard University,
Cambridge, Massachusetts 02138
and Department of Biological Sciences,
Purdue University,
West Lafayette, Indiana 47907*

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FLAMING AND BROWN REPLY—in agreement with all other authors, Lipton *et al.*[1] found that increased extracellular Ca^{2+} markedly reduced V_{max}. Their new finding was that raised or lowered extracellular Ca^{2+} concentration exerted no detectable effect upon σ, the stimulus intensity eliciting a response of half the maximum amplitude, although σ was altered significantly by adaptation. They stated that "The experiments do indicate that in a partially light-adapted rod the membrane potential and cytosol Ca^{2+} levels do not regulate the major determinant of sensitivity (σ), ...". We recently[2] showed that when external Ca^{2+} was varied from 0.3 to 6.0 mM, σ shifted to higher light intensities by about 0.75 log units. Both increases and decreases of Ca^{2+} concentration from the control level of 1.8 mM elicited consistent σ shifts that were detected readily. Our conclusions were: "Thus, our results indicate that alteration of $[Ca^{2+}]_o$ can control the value of σ in the V–log I curve of toad rods. These results are consistent with a role for $[Ca^{2+}]_i$ in the control of sensitivity in vertebrate photoreceptors. Hence, changes of $[Ca^{2+}]_i$ may partially control the changes of sensitivity that occur during light and dark adaptation". Our conclusion that $[Ca^{2+}]_o$ can exert a controlling effect on σ differs from the conclusion of Lipton *et al.*[1] in an important respect, and we note that they now accept the validity of our findings on this point.

One remaining question concerns the degree to which $[Ca^{2+}]_i$ can control the value of σ. Taken at face value, our results suggest that $[Ca^{2+}]_i$ could only control a small portion of the adaptive σ shifts. However, we demonstrated that our Ca^{2+}-induced σ shifts depended on several improvements in experimental conditions, and one critical factor was a more normal perfusate containing 15 mM of bicarbonate, which was used for buffering[2] instead of HEPES[1]. It thus seems that the induction of σ shifts by altering $[Ca^{2+}]_o$ is more sensitive to subtle chemical factors in the perfusate than are the effects of $[Ca^{2+}]_o$ on V_{max}. This suggests that it is especially important to compare σ shifts induced by adaptation, and by alteration of $[Ca^{2+}]_o$, in physiologically normal experimental conditions. Although we have made some improvements in that direction, we are not convinced of having attained the requisite success, which may be very difficult with an isolated and perfused preparation. Also, even in a completely normal preparation, an elevated $[Ca^{2+}]_o$ may not adequately mimic the effects of increased $[Ca^{2+}]_i$. For reasons such as these, we deliberately drew no conclusion concerning the degree to which $[Ca^{2+}]_i$ may normally control adaptive σ shifts. Although Lipton *et al.*[1] failed to detect any of the controlling effect of $[Ca^{2+}]_o$ on the value of σ, their letter indicates great confidence concerning the quantitative control of σ by $[Ca^{2+}]_i$. As premature conclusions based on inadequate evidence can be 'misleading', it seems advisable to await more definitive evidence on this issue.

DALE G. FLAMING
KENNETH T. BROWN

*Department of Physiology,
University of California,
San Francisco, California 94143*

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reviews

Deficiencies in the Green Revolution

D.L. Gunn

Beyond the Green Revolution: The Ecology and Politics of Global Agricultural Development. By K.A. Dahlberg. Pp. 256. (Plenum: New York and London, 1979.) £11.30.

MILLIONS of people are undernourished or even starving, especially in the tropics. Yields of basic foods, generally cereals, are often poor. The Rockefeller Foundation therefore sent a team led by N.E. Borlaug into Mexico to breed more productive varieties of wheat for use in the tropics. He was very successful and the new seed has much increased crops in many places, for example enabling Pakistan to change from importing to exporting wheat. The technique has since been followed with other cereals. This has been called the Green Revolution, the great increase in food using scientific means.

Everyone involved in agriculture knows, however, that large increases in crop yields cannot be achieved and sustained by simply substituting the new seed for the old. More plant food (artificial fertiliser), extra water, herbicides and insecticides may all be required. Thus, full success with new wheat strains requires money to be invested in these extra inputs and also new knowledge and skills. The vast numbers of struggling subsistence farmers — about whom Dahlberg is mainly concerned — are too poor to use the new wheat.

Kenneth A. Dahlberg, a social scientist, has come across these well-known facts and in this book, emphasising the deficiencies of the Green Revolution, he pleads for re-assessment of situations by "contextual analysis". For biologists there is nothing new in seeking to examine a situation as a whole before suggesting how to alter it; that is similar to examining a problem in its context. Dahlberg explores a very wide context. In three chapters entitled "Ecology of Theories", "Historical Seedbeds" and "Jack and the Beanstalk", he outlines the influence of European tradition, of climate on the kind of ecological theory produced, and of trade on economnic thought; the resulting ideas have little relevance to subsistence agriculture in the tropics. Contextual analysis is put forward for examining situations instead of proposing actions merely because they would be effective in a temperate climate.

The remaining chapters are headed "The Momentum of Structures", "Institutions and Current Policies"; "New Approaches to the Future"; "Agricultural Strategies and Policies for the Future"; and "Hindsight, Insight and Foresight".

The author is critical and censorious about the European colonial powers, especially the British, to whom he imputes selfish national motives. Actually many agricultural officers and district commissioners were more concerned about the welfare of the local people than about the trade interests of the mother country. They discovered most of the complications that Dahlberg expounds and their long and devoted service did help the peasants in many places; but they did not solve the problem of financing innovation — not merely a matter of providing the money. The British Government set up the College of Tropical Agriculture in Trinidad, through which intending agricultural officers passed. They were trained to understand both agricultural and sociological complications, either in the College or on the job.

Amongst the many criticisms of European influence, there is one commendation implied in the stated failure of North American charities "to draw upon the extensive experience of Europeans in semi-tropical research, extension and education"; but why *semi*-tropical?

There is of course no doubt that European influence in tropical countries has disrupted old ways, often regretably; but should the destruction of the Ashanti, the Zulu and the Matabele bloodthirsty modes of life be regarded as good or bad? Those tribes certainly restricted the population growth of others; and the Zulus left much of the Transvaal empty and open to the incoming Boers.

Dahlberg does mention the explosive increase in human population as the most important change now taking place, but he merely asks "Can you condemn millions to starvation by not making every effort to increase agricultural production, especially if there is any chance that some solution to the population problem can be found in coming decades?" He hopes for options other than "lifeboat ethics".

It is this population increase in the less developed countries — and only there — that creates the problem. There would be little difficulty today in feeding the world population of my youth, two thousand millions, but where will it end? Well-intentioned Christian missions, the pax Britannica, medico-sanitary innovations by Europeans in the tropics, saving the children, and famine relief in general, have been responsible for creating the problem and destroying long-term stability. The problem is not how to produce enough food for everyone at present, although that is how it is usually stated. The problem is how those who cannot produce enough for themselves are to pay others to produce it for them. It is a problem of poverty. What can the poor do or produce that the rest of the world will be willing to buy? Industrial development, rejected by Dahlberg, is one partial solution, although it increases the problem of urban poverty, constantly fed from a profligately breeding rural population. Producing enough food for everyone now, even if it has to be given away to the poor, merely postpones greater starvation. There is no remedy in the long term for food shortages and for losses of irreplaceable resources without stopping the growth of population.

The author cites the resistance of the developing countries at the 1972 Stockholm Conference to any interference by environmentalists (regarding, for example, use of pesticides) and interprets it as misplaced confidence by elitists in Western conceptions of development; it seemed to me to arise more from preference for preserving the lives of their people rather than their eagles. That was certainly expressed at the Royal Society Discussion on Control of Migrant Pests in 1977. He also refers to vested interests in the debate over pesticides, without mentioning specifically the large and lucrative anti-pesticide industry, which was prominent at Stockholm. He writes that the World Food Conference and other such planning meetings propose solutions to food supply problems that are anti-ecological, evidently using the word ecological in the modern propaganda sense rather than as it is used in biology. Indeed, his acceptance of alarmist propaganda makes one wonder how much reliance is to be placed in statements in less familiar spheres.

This book is well-intentioned, sometimes interesting, sometimes dreary, sometimes misleading, sometimes muddled — "What is needed are a series of policies...", and sometimes obscure —

"information forwarded by life...". A more objective and perhaps humble analysis of past searching for improvement, less censorious but critical where something could be learnt, would have been more acceptable. It would have been illuminating to have a comparison of progress in Africa with that in South America. The book may have something for social scientists; it has nothing for agriculturalists.

There are nine pages of references and an index of 18 pages. In neither is Borlaug's name mentioned. ☐

D.L. Gunn, now retired, has been Director of the International Red Locust Control Service, Northern Rhodesia, Director of the Tea Research Institute, Ceylon, and Adviser to the Secretary of the Agricultural Research Council, London, UK.

African mammal palaeontology

Evolution of African Mammals. Edited by V. J. Maglio and H. B. S. Cooke. Pp.641. (Harvard University Press: London and Cambridge, Massachusetts, 1978.) £42; $60.

POTENTIAL READERS should clearly appreciate that this volume represents the first effort to put together in one book complete overviews of all African mammal groups. I want to congratulate the editors, Maglio and Cooke, for providing the impetus which led each of many specialists to articulate for the first time the entire known African story of his group. It should be stressed that much of what is presented in this volume, both in terms of materials and discussion, is quite new.

Authoritative introductions on present-day mammals of Africa (Bigalke) and the geological development of the physical African setting (Cooke) set the stage for 27 systematic chapters. These vary greatly, and appropriately, in length and depth of treatment. There can be little doubt but that the 95 pages by Howell represent the most complete and useful treatment available of the African evolution of the family of man, the Hominidae. The paper combines some provocative interpretations with admirably balanced consideration not only of the different African fossil sources, but also of the legion of opinions expressed on and around hominid evolution.

Several other larger contributions are particularly impressive and comprehensive, notably those on Proboscidea (Coppens, Maglio, Madden and Beden), Bovidae (Gentry), Suidae and Tayassuidae (Cooke and Wilkinson) and Equidae (Churcher and Richardson). There are also substantial chapters on mesozoic mammals (Crompton and Jenkins), Insectivora and Chiroptera (Butler), Rodentia and Lagomorpha (Lavocat), prosimian primates (Walker), Cercopithecidae and Parapithecidae (Simons and Delson), cenozoic apes (Simons, Andrews and Pilbeam), *Ramapithecus* (Simons and Pilbeam), Carnivora (Savage), Pholidota and Tubulidentata (Patterson), Hyracoidea (Meyer), Deinotherioidea and Barytherioidea (Harris), Rhinocerotidae (Hooijer), Anthracotheriidae (Black), Hippopotamidae (Coryndon), Cervidae and Palaeomerycidae (Hamilton), Giraffidae (Churcher), Sirenia (Domning) and Cetacea (Barnes and Mitchell).

Some shorter papers on lesser known groups add fascination by the strangeness of the beasts they describe: Embrithopoda (Tanner), Moeritherioidea (Coppens and Beden), Chalicotheriidae (Butler), Tragulidae and Camelidae (Gentry). Maglio's summary chapter on faunal turnover and succession, distribution and endemism, will probably be extensively used in future zoogeographic and evolutionary studies.

Much as each of the 30 fine contributions in this volume deserves individual comment, in a review of this length I will have to be content with expressing some overall impressions. The chronology, geography and stratigraphy of fossil finds are exceptionally completely and lucidly covered in numerous tables, maps and discussions. The histories of early fossil finds and conclusions are full of interest, and occasional humour. For example, in Harris' work on deinotheres we are told: "Buckland's (1835) quaint portrait of a mammal anchored to a river bank by its tusks while it slept preceded the discovery of deinothere hind limbs". Illustrations pinpointing precisely salient morphological differences or primitive-to-advanced series, like those provided by Gentry (p544), Crompton and Jenkins (pp45 and 50), Hamilton (p506) and others, are valuable in a reference book of this sort. It is regrettable that the book contains too few of them.

Bibliographical coverage is excellent, with only a few exceptions (for example, the lack of reference to White and Harris' (1977) major work on fossil suids in the chapter by Cooke and Wilkinson; omission of several papers on African Carnivora, for example, of M.G. Leakey, Howell and Petter; and only cursory reference to important contributions by other carnivore specialists, such as Hendey and Ewer, in the contribution by Savage; and because the phylogenetic status of *Ramapithecus* is by no means established, as Simons and Pilbeam readily admit in their paper on this taxon, the inclusion of a larger bibliography and debate pro and contra hominid status of *Ramapithecus* would have further added to the great interest of this chapter).

In several instances good reviews of past and ongoing debates make exciting reading, as in the discussion of cetacean origins and the phylogenetic position of the African Archaeoceti by Barnes and Mitchell. Fortunately for a palaeontological *tour de force* of this length several of the authors have the ability to bring their long-dead creatures to life again. The expression of such ability, from deliberations on brain size and locomotion in the earliest mammals (Crompton and Jenkins) and on the evolution of the ant-eating habit in aardvarks (Patterson), to questions about how deinotheres used their tusks (Harris) and about why sirenians speciate slowly (Domning), adds greatly to a real enjoyment of this book.

In the preface the editors remark: "Such overviews (as this one) are the only means of . . . picking out our significant achievements and failures". On reading through the whole book others too may perceive, as I do, a disturbing failure common to a sizeable block of the present contributions. On the one hand many morphological details are given; on the other, conclusions on relationship (in some cases 'trees') are presented. But between these two there is often an hiatus; that is, it is difficult, at best, to extract precisely which morphological details are considered by the author pro, and which contra, his hypotheses of relationship. The reader is thus unable to test such hypotheses. In individual cases it is not clear whether the author has preferred to base his conclusions only on unsorted impressions; or whether he *has* used a particular methodology to arrive at clear statements supporting his conclusions, but has merely failed to articulate these statements to his readers. Several of the papers stress the paucity and fragmentary nature of the fossils. That such shortcomings do not justify a lack of methodology and clarity is shown by the fact that some contributors on the poorest fossil samples (for example, Crompton and Jenkins on Mesozoic mammals, Hamilton on Cervidae and Palaeomerycidae, Patterson on Pholidota and Tubulidentata, and others) are among those that make the clearest statements in support of their hypotheses of relationship.

This large, sturdy volume, in which printing, reproduction of illustrations, binding and indexing are all of high quality, is unique in fully representing present knowledge of African fossil mammals. Whether or not one's own interpretations (and indeed mode of interpretation) differ from any of those in *Evolution of African Mammals*, there is no better companion than this book for anyone, whether student or specialist, who is interested in African mammal palaeontology. **E.S. Vrba**

E.S. Vrba is Head of the Department of Palaeontology and Palaeoanthropology at the Transvaal Museum, Pretoria, South Africa.

Plastic crystals

The Plastically Crystalline State: Orientationally-Disordered Crystals. Edited by John N. Sherwood. Pp. 383. (Wiley: Chichester, UK, and New York, 1979.) £19.50.

THIS is a valuable and interesting book, dealing with a type of crystal to which increasing attention has been paid in recent years. Plastic crystals are frequently rather arbitrarily defined as those for which the entropy of fusion is less than ~ 20 J K^{-1} mol^{-1}, but the editor wisely decided that his contributors should not be rigidly confined by this limitation, and references are made to orientationally disordered solids not satisfying this entropy of fusion criterion, and occasionally also to salts and to liquid crystals.

The book is essentially a collection of essays by experts on some of the techniques available for investigating orientational disorder in crystals, and especially of newer methods still in the evolutionary stage. There are no accounts of certain techniques which, though admittedly of longer standing, will nevertheless continue to be used, such as experimental thermodynamics and neutron diffraction. The topics covered include methods of studying self-diffusion, diffuse X-ray scattering, dielectric and acoustic studies, NMR, Rayleigh and Brillouin scattering, infrared and Raman spectroscopy, and neutron scattering. There are also chapters on the structures of plastic crystals, on the correlation of dynamic observations of molecular motion, and on the theoretical aspects of orientational disorder. The quality of exposition is generally high, and the text is illustrated by well chosen figures.

A typical chapter describes the theory and practice of the technique with which it is concerned, and there then follow examples of its application to particular systems. In consequence, the sum total of the space devoted to any particular crystal can vary considerably. Thus, there are 29 references in the index to adamantane, 35 to cyclohexane, and 23 to hexamethylethane, which in each case are distributed fairly uniformly throughout the book. On the other hand, there is very little on the plastic crystalline phases of nitrogen, oxygen, carbon monoxide and fluorine, even though these have received a good deal of attention on account of their comparative molecular simplicity.

When this book has so much to commend it, it is a pity to have to add that it contains more minor misprints and mistakes than one might have expected to find. However, these are minor blemishes on a book which should be a real asset to those actively interested in the subject.

LA.K. Staveley

L.A.K. Staveley is Fellow and Tutor of New College, Oxford, and University Reader in Inorganic Chemistry at the University of Oxford, UK.

Control of movement and posture

An Introduction to Physiology. Vol.4. By H. Davson and M.B. Segal. Pp.573. (Academic: London; Grune and Stratton: New York, 1978). £11.80.

THE latest volume in this impressive series is devoted to the control of movement and posture, which it treats in considerable depth. The first section begins with an instructive general account of quadrupedal locomotion which, as much experimental work is performed on four-footed subjects, is clearly justifiable on scientific grounds, but may not be thought wholly relevant by readers primarily interested in man.

This is followed by a brief discussion of the grading of muscular effort and a detailed description of proprioceptive sensors leading on to a consideration of spinal reflexes, which starts from the work of D.P.C. Lloyd in 1943 rather than, as might have been expected, from that of Sherrington. In this chapter the contributions of the Matthews, father and son, to spindle-physiology, together with modern views on the significance of the tendon organs, are surveyed.

The second section of the book is concerned with supra-spinal control of motor activity and contains chapters on the vestibular apparatus, cerebral cortex, cerebellum, reticular, olivary and red nuclei, and also the basal ganglia. The final chapter comes full circle and returns to broader aspects of the coordination of movement. In dealing with topics as complex as these it is immensely difficult to create for the first-time reader (for whom, presumably, an 'introduction' is intended) a clear and coherenet outline but a brave effort has been made and, on the whole, successfully.

The text is conveniently subdivided by frequent and informative headings and is copiously illustrated and documented with references. Most of the illustrations display the experimental results obtained by the investigators whose work is discussed while the remainder are explanatory diagrams.

My assessment of the status of this multi-volume treatise was incorporated in the review of an earlier volume see (*Nature* **266**, 284; 1977) and remains unchanged.

R.V. Coxon

R. V. Coxon is Reader in Human Physiology at the University of Oxford, UK.

Published today are the two latest additions to the *Contemporary Biology Series:*

An Introduction to Evolutionary Genetics
David T. Parkin

The author shows how an understanding of the genetic structure of populations and the forces that interact with this can produce evolutionary change. Introductory sections on metric characters and molecular evolution and a wide range of examples from experimental and field studies assist in developing the principles of how evolution can occur as a result of gene substitution.

This is a topic of great biological importance, but it is often considered theoretically rather difficult. In this book, Dr Parkin presents and discusses the genetic basis of evolution and speciation in a clear way, maintaining a careful balance between the ecological and the theoretical aspects of the subject.

Paper £6.50

The Biology of Parasitism

An introduction to the study of associating organisms
Philip J. Whitfield

This new textbook of parasite biology avoids the traditional view of parasitism as a discrete way of life and shows convincingly that it is an often arbitrarily defined part of a broad spectrum of associations between organisms. In doing this, Dr Whitfield rejects the taxonomic approach and instead organises his material around themes such as nutrition, physiology and regulation, behaviour, and ecology and evolution.

Important advances have been made in many of these areas over the past decade. The use of new technologies, such as scanning electron microscopy, and new conceptual approaches, such as the application of rigorous population dynamical theories to association biology, have contributed largely, but many of these developments have not, until now, been reported in undergraduate texts.

Dr Whitfield has used this new material most effectively in his carefully distilled arguments by concentrating on thoroughly researched examples. His style is clear and lively and the writing is supported by excellent diagrams and electron micrographs.

Paper £7.95

Edward Arnold
41 Bedford Square,
London WC1 3DQ

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Stellar evolution

Stars and Clusters. By Cecilia Payne-Gaposchkin. Pp. 261. (Harvard University Press: Cambridge, Massachusetts, and London, 1979.) £13.50; $22.50.

PAYNE-GAPOSCHKIN was in no small way responsible for my deviation from the straight and narrow path of pure physics to the ethereal delights of astronomy. I devoured her book *Introduction to Astronomy* (Eyre and Spottiswoode, 1956) during my second year at university and never looked back. That book, like *Stars and Clusters*, is intended for the student and the general reader who may have little background in mathematics or physics. To this end *Stars and Clusters* is written without recourse to any mathematical formula; not a single equation graces its pages. Our inumerate unphysical friend can therefore romp through the text with ease.

But what about the figures? I was immediately struck by the fact that the figures must be considerably more difficult to understand than the text if you didn't "have the mathematics". The intricacies of the Hertzprung-Russell diagram especially when it contains multiple stellar evolutionary tracks are difficult enough to grasp at the best of times. Our non — mathematical student has probably never drawn a graph in his life, has no idea what a logarithm is and must be confused by the unexplained leap from (B—V) to log surface temperature for the abscissa without the added complication that the later scale is inverted. It is highly laudable to write a book with the non — mathematical student in mind, but if this student is to gain most from the book I think he must be taught some maths on the way; he must, to quote Martin Johnson, "discover for himself how little armoury he needs for appreciating the beauty and convincement of tracing an argument in symbols and actual quantities".

Stars and Clusters falls between two stools. The inumerate student would appreciate help with the graphical concepts and the numerate student would benefit greatly from a mathematical treatment. This of course isn't to say that I did not enjoy reading the book. I did. But I was constantly reminded that one small equation must be replaced by many and complex words if the same message is to be conveyed.

The main theme of the book is the effect of evolution on stellar characteristics, the physical processes underlying stellar evolution and the light thrown on to this problem by the observation of open clusters, globular clusters and variable stars. The book is impressively illustrated and it is obvious that enormous care has been taken in the choice of the figures. An indepth study of the Hyades cluster in Taurus is used to introduce the early stages of stellar evolution, and one of the figures is a plot of apparent visual magnitude as a function of (B—V) colour for all the stars in the Hyades field of view. The interpretation of this figure would be a fascinating problem for a first-year astronomy student at university. The same can be said of a figure in the next chapter, the path of a metal rich star of five solar masses on the Hertzprung-Russell diagram. I have the sneaking suspicion that both these would leave the amateur astronomer way behind. The book contains about thirty figures showing photographs of clusters and absolute magnitude against colour plots of their stars; again a fascinating study for the university student. A careful distinction is made between the evolution of single stars and the peculiar mutual influence that double stars exert on each other and on their evolution. The book contains a superb collection of light curves for variable stars; one indication of its thoroughness is that there are 48 for R.R. Lyrae stars alone! The AAVSO star atlas is included at the end of the book so if you want to find, for example, X Geminorum you are shown where to look.

I am going to recommend this book very strongly to my first-year university students. They will be able to understand the figures, and will not need all the footnotes that rather verbosely explain the simple physical concepts. It may seem strange but the fact that it contains no mathematics makes me reluctant to recommend it to an inumerate student. Many of the concepts introduced and most of the concepts illustrated need a more detailed and also a more numerate explanation to make then understandable.

David W. Hughes

David W. Hughes is Lecturer in Astronomy and Physics in the Department of Physics at the University of Sheffield, UK.

Conidium ontogeny

Patterns of Development in Conidial Fungi. By G.T. Cole and R.A. Samson. Pp. 190. (Pitman: London, San Francisco and Melbourne, 1979.) £29.75.

SINCE S.J. Hughes in 1953 firmly placed developmental characteristics of conidial fungi amongst the prime ones for classifying Fungi Imperfecti, studies of conidium and conidiophore ontogeny have galloped apace. There is no doubt that these researches have generated abundant new taxonomic criteria or have illumined established ones. The difficulty has largely been in judging the significance of these characteristics. The fact that a number of attempts have been made to erect more or less formal classifications based primarily on features of conidiophore and conidium development shows that conidial fungi undoubtedly *can* be classified by means of these criteria, but what is less clear is whether such classifications reflect the phylogeny of the organisms concerned.

It is because the taxonomic significance of ontogenetic data is still an area of lively controversy that this new book is such a timely and valuable contribution. The authors have brought together an enormous amount of information, chiefly in pictorial form and much of it original (mainly as new scanning electron micrographs), so that the theorising mycologist who wishes to reclassify the fungi and the morphogeneticist who wants to find how fungi come to look the way they do have very little need to look at real live fungi — at least, while they are planning how best to go about their studies. The quantity of factual information is so large and the authors' presentation so good that virtually all that is needed for armchair speculation is available here in a single convenient volume. The authors themselves are reticent about the formal taxonomic implications of the book's contents. They are explicitly and unambiguously concerned with patterns of conidial development, and it is these which they classify, not the fungi themselves.

Two-thirds of the book deals with various modes of conidium ontogeny in Fungi Imperfecti, chiefly hyphomycetes. The remainder includes an account of techniques for studying the development and ultrastructure of conidial fungi. Because so many of the electron micrographs presented are original it was wise of the authors to have included this chapter which effectively amounts to a 'materials and methods' section. There is also a major section on patterns of sporangiospore development in Zygomycetes which serves to illustrate how another group of mycelial organisms has solved problems similar to those facing higher fungi. A glossary eases the way for those not familiar with the subject's specialised terminology.

The text is copiously and beautifully illustrated and astonishingly free from printing errors. The authors write well (that is, they say precisely what they mean), and though they use specialised terms where appropriate, they eschew jargon. The high quality of the book's format is reflected in its price, but despite this it is likely to appear in most mycology teaching libraries, as it furnishes in convenient form so many data and concepts. Researchers in the field of fungal taxonomy and developmental biology will use it for these reasons and as a source of esoteric pleasure.

M.F. Madelin

M.F. Madelin is Reader in Mycology at the University of Bristol, UK.

CENTRALLY ACTING PEPTIDES
Edited by J. Hughes

A Biological Council Symposium on Drug Action

The April 1977 symposium was stimulated by the dramatic increase in research into functionally active peptides in the central nervous system, and the discovery of new peptides such as neurotensin, the enkephalins and the endorphins. All aspects of research – biochemical, electrophysiological and behavioural, as well as radio-immunoassay and radiofluorescence – are covered in this volume, which also points out the many gaps that still remain in our understanding of the subject. It is thus a timely review of the current state of research, and will be invaluable to anyone involved in any aspect of peptides.

Contents
Isolation and Identification of Biologically Active Peptides; H. R. Morris
Immunohistochemical Localisation of Peptides in the Nervous System; R. Elde, T. Hökfelt, O. Johansson, A. Ljungdahl, G. Nilsson & S. L. Jeffcoate
Are 'Hypothalamic Hormones' Central Neurotransmitters?; M. J. Brownstein
Diffuse Neuroendocrine System: Peptides Common to Brain and Intestine and their Relationship to the APUD Concept; A. G. E. Pearse
Electrophysiological Analysis of Peptide Actions in Neural Tissue; L. P. Renaud & A. Padjen
Comparative Features of Enkaphalin and Neurotensin in the Mammalian Central Nervous System; S H. Snyder, G. R. Uhl & M. J. Kuhar
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Physiological and Clinical Relevance of Endorphins; L. Terenius & A. Wahlström
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Monographs for Students of Medicine

Control of Human Reproduction

R. L. Holmes and C. A. Fox

October/November 1979, viii + 150pp., £5.50/$12.50 0.12.353450.X

Control of Human Reproduction aims to bridge the gap between the basic sciences and clinical knowledge of the endocrinology of reproduction. The authors present a general survey of the role of hormones in reproductive processes with particular reference to the human system, giving the anatomical, physiological and chemical facts and their clinical applications. The book gives a clear exposition of the reproductive hormones, the tissues which produce and control them, and the way in which a variety of factors bring about the development of functional reproductive systems and influence them in maturity. The mechanisms of reproduction and Man's ability to control his own numbers and to affect his environment are considered, as well as abnormalities of development and disorders of reproduction. The combination of a basic approach with clinical applications makes the book of value to medical students throughout their course and to practising clinicians.

Aircrew Stress in Wartime Operations

edited by E. J. Dearnley and P. B. Warr

October/November 1979, vii + 224pp., £8.50/$20.00 0.12.207750.4

This is a book of important research papers in psychology and psychiatry. They were first published between 1940 and 1948, being sponsored by the Flying Personnel Research Committee of the Ministry of Defence. They are of major historical significance, but the current upsurge of interest in the sources and management of stress ensures their topicality. Many themes and methodological features are of central importance for today's investigators. The papers describe eight research projects to examine the performance of aircrew during the second world war. The projects range from statistical investigations of accidents and effectiveness, through more clinical studies of personality and individual functioning, to the classic psychological experiments into fatigue and pilot error. The eight research reports are preceded by a specially written account of the historical background to the studies. The book provides a fascinating insight into developments during a period when behavioural research achieved considerable advances.

Handbook of Microbiological Investigations for Laboratory Animal Health

J. R. Needham

October/November 1979, xviii + 174pp., £9.80/$21.00 0.12.515950.1

The information presented in this book is based principally on microbiological techniques employed by the author at the Clinical Research Centre in their animal health programme. This scheme is described in the book in full detail with its various stages set out together with the laboratory tests necessary to monitor animal health. Each chapter describes the application and results of tests used for animals including mice, dogs, farm animals and subhuman primates; thus the animal health worker is provided with data than can be compared with his own findings. In addition, the details are included for several experiments which were carried out in a search for alternative methods. Designed as a reference work and bench book for microbiologists and animal health scientists, this book should provide an invaluable guide for all institutions which make use of animal laboratories for agricultural, medical, commercial or veterinary purposes.

Surgical Sepsis

edited by C. J. L. Strachan and R. Wise

October 1979, x + 154pp., £11.80/$25.00
0.12.794378.1 (Academic Press)
0.8089.1208.9 (Grune & Stratton)

The symposium, of which this book is the proceedings, was designed to provide a platform for the review and discussion of methods currently in vogue for the prevention of abdominal wound sepsis. The speakers were chosen for the recent contributions they have made advancing the state of knowledge concerning the factors involved in wound sepsis, and included one of the 'fathers' of surgical sepsis research in the U.S.A. — Professor Harlan Stone. Their topics range from the mundane 'hot appendix' to the more esoteric and, until recently, poorly understood syndrome of pseudomembranous colitis, trials of wound guard, wound sprays or instillates of topical bacteriocides. The text will undoubtedly benefit surgeons and microbiologists as well as medical students and workers within the pharmaceutical industry, and is likely to provide the standard reference work for the next decade.

Language, Thought and Culture: Advances in the Study of Cognition

Classifications in their Social Context

edited by Roy F. Ellen and David Reason

October/November 1979, xii + 262pp., £13.00/$30.00 0.12.237160.7

The comparison of different forms of classification raises fascinating and fundamental questions. Is the tendency to classify the result of the way in which we process information (a logical or material imperative), or is it the result of more localized pressures (a social or historical imperative)? Do the terms, logic and structure of these forms of classification reflect the logic and structure of a particular society, or do they mould the social environment so that it too may become comprehensible? Do we discriminate socially because we are bound to classify in a discriminatory way, or vice versa? This book approaches these problems in two ways. The majority of the papers draw on examples of 'folk' and 'scientific' classification in a specific ethnographic or historical context. Together they provide a comparative approach that allows the remaining papers to relate the problems of understanding classification to current debates in anthropology and sociology, especially concerning the relationship between knowledge, symbolism and socio-economic activity.

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Quantitative Cytochemistry and its Applications

edited by J. R. Pattison, L. Bitensky and J. Chayen

October/November 1979, xvi + 322pp., £14.00/$32.50 0.12.546750.8

A two day international meeting was held in London on November 13 and 14, 1978, supported by the Section of Pathology of the Royal Society of Medicine, entitled "Quantitative Cytochemistry and its applications". This volume, which is published in relation to, and has taken the same title as, this meeting, presents revised and expanded papers dealing with the range of applications of quantitative cytochemistry to medical research. Although the methods used in such studies are described in some detail, emphasis is placed on application and the relevent information obtained rather than on the minutiae of techniques; full references are given for those who wish to study the methodology in more detail. This is the first time that the diverse applications of this subject have been collected together in one volume demonstrating that the results obtained by cytochemical methods yield valuable information and useful answers in medicine, toxicology and biochemistry.

A.P.I.C. Studies in Data Processing No. 15

Computer Architecture: A Structured Approach

R. W. Doran

October/November 1979, x + 234pp., £14.20/$30.00 0.12.220850.1

In this book the author adopts a new approach to the subject, discussing the principles behind computer architecture and at the same time illustrating these principles by developing a detailed description of one machine, the Burroughs B6700. The first part of each chapter is spent in building a general model of what the architecture of a general purpose computer is designed to achieve, laying down the various functions in easy stages to work up gradually to the full complexity of the machine. In the second half of each chapter the functional architecture of the B6700 is described and assessed in terms of the model. In general the text assumes a fairly broad knowledge of computers though not of computer architecture itself, and does not hesitate in going back to basics where necessary to clarify a principle. This book provides an ideal introduction both to the principles of computer architecture and to how they are implemented.

Interaction within the Brain Pituitary Adreno-Cortical System

edited by M. T. Jones, B. Gillam, M. F. Dallman and S. Chattopadhyay

October/November 1979, c280pp., £14.80/$34.50 0.12.389150.7

The book is based on a satellite symposium of the Vth International Congress in Hormonal Steroids held at Varanasi, India, in November 1978. The contributions are representative of the broad range of experimental strategy and clinical observations that are being used to further our understanding of a complex interacting system. Although due prominence is given to recent discoveries concerning the corticotrophin-related and ß-lipotrophin-related peptides, care was taken to ensure that aspects of the subject that are frequently neglected were adequately covered. The scope of the book is well reflected by the range of specialists who have contributed chapters, including endocrinologists, anatomists, clinicians, pathologists, physiologists, neurologists, pharmacologists, biochemists, behavioural scientists and developmental biologists.

Pasture and Politics

Economics, Conflict and Ritual among Shahsevan Nomads in Northwestern Iran

Richard Tapper

October/November 1979, xvi + 312pp., £17.00/$39.50 0.12.682660.4

Questioning the validity of stereotypes, the author examines the relation between grazing rights, local community politics and ritual behaviour among the Shahsevan nomads of northeastern Azarbayjan, in Iran. In the first chapter of the book the author underlines the importance of these issues for comparative and theoretical study, while the final chapter places the Shahsevan in a broader perspective by comparing them with some other societies, and draws some general conclusions about the relation between territorial and political organisation. The author's unusual material and approach will be of considerable interest to teachers, researchers and students in the fields of social anthropology, cultural geography, Middle Eastern and Islamic studies, and development economics.

Environmental Chemistry of the Elements

H. J. M. Bowen

November/December 1979, xvi + 334pp., £17.00/$39.50 0.12.120450.2

This book aims at a comprehensive and critical rather than encyclopaedic summary of the data of environmental inorganic chemistry. The first four chapters deal with the air, water, rocks and soils, and stress the dynamic nature of elemental cycling in these media. The cycling of the organic elements carbon, hydrogen, oxygen, nitrogen and sulphur in the biosphere has a chapter to itself. The elemental composition of living matter is reviewed with the help of tables of selected data. Other chapters are concerned with the uptake and excretion of elements by plants and animals, together with criteria for essentiality, toxicity and deficiency. Two chapters cover biological fractionation of isotopes, and the occurrence of natural and artificial radionuclides in the environment. Environmental disturbances through human activities are summarised in the penultimate chapter. A special feature is the last chapter which reviews the date available for each of the elements in turn.

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The Institute of Mathematics and its Applications Conference Series

Mathematical Aspects of Marine Traffic

edited by S. H. Hollingdale

October/November 1979, xii + 270pp., £17.50/$40.50

This book contains papers that were presented at a two-day conference held at London University and organised by the Institute of Mathematics and its Applications in collaboration with the Royal Institute of Navigation and several other organisations. The two-fold purpose of the conference was; to bring marine traffic to the attention of applied mathematicians as a rewarding field of research, and to inform marine navigators and technologists of the varied contributions that a mathematical approach can make in their field. The contents of this book bear witness to the importance and versatility of mathematical models in marine traffic studies, and also to the current pre-occupation with routeing schemes. The papers will be of professional interest and great assistance to applied mathematicians and marine engineers working in government and industry and also of educational use to undergraduates and teachers.

Comparative Physiology of Osmoregulation in Animals

edited by G. M. O. Maloiy

Volume 1

October/November 1979, xii + 678pp., £43.50/$91.50 0.12.467001.6

Volume 2

November/December 1979, xii + 246pp., £18.60/$43.00 0.12.467002.4

In these two volumes much work has been brought together in comparing the special adaptive mechanisms that have evolved in a variety of animals for solving the physiological problems facing them in maintaining ion and water balance. The general principles underlying osmoregulation are discussed, as well as specific regulatory systems found in different groups of animals, each chapter dealing with a separate group with reference to particular environments. In addition, the mechanisms of ion transport occuring in may tissues are considered, with detailed accounts of some important cases including kidney tubules, malpighian tubules, fish gills and nasal salt glands. Few researchers can be expert in all these areas, from fresh water invertebrates to desert camels, but with these volumes they now have access to the latest developments in the field.

Computational Mathematics and Applications

Stable Recursions

With applications to the numerical solution of stiff systems

J. R. Cash

November/December 1979, c220pp., £19.60/$45.50 0.12.163050.1

The main aim of this book is to establish a relationship between the theories of linear recurrence relations and ordinary differential equations and to exploit this relationship to produce new algorithms for the numerical integration of ordinary differential equations. This oook is not intended as an introduction to finite difference methods, but it is hoped that the material presented wil help the reader to deepen his understanding of this particular class of methods. It is a research work with many open ended questions and includes several sections which are supposed to stimulate further research. It also shows that many of the currently used methods are particular cases of much larger families of methods, which are as yet unexploited. For these reasons the book should be of interest to anyone involved in the numerical solution of differential equations.

The Molecular Physics of Liquid Crystals

edited by G. R. Luckhurst and G. W. Gray

October 1979, xiv + 494pp., £20.00/$46.00 0.12.458950.2

This book has its origins in a NATO Advanced Study Institute whose primary aim was to clarify the present situation by discussing both theoretical and experimental approaches used to study liquid crystals at the molecular level. The result is not only a unique reference work but, by virtue of the original research described, a stimulus for further investigation. Among the experimental techniques discussed are magnetic resonance, spectroscopy, neutron, X-ray and light scattering, as well as thermodynamic measurements. The statistical mechanics necessary to understand the theory of liquid crystals are reviewed and then used to consider molecular-field and hard-particle theories of nematics and cholesterics. This book is not aimed exclusively at physicists and chemists, but contains much that is of value to engineers, applied physicists, electronic engineers and pure theoreticians.

Beta-lactamases

edited by J. M. T. Hamilton-Miller and J. T. Smith

October/November 1979, xii + 500pp., £26.40/$61.00 0.12.321550.1

The Editors of this book, both of whom have been actively involved in this topic since the introduction of the first semisynthetic penicillins, have tried to bring together some important aspects of ß-lactamase, both conceptual and practical. The chapters should be of interest to a very wide audience, and will provide an overall view of our present understanding of this important group of enzymes. Not only research workers in Universities and Industry, but also members of hospital departments of Medical Microbiology, Pathology and Pharmacology, and biochemists, immunologists, geneticists and molecular biologists, will find much of lasting and practical interest to them in this volume.

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Soil-Borne Plant Pathogens

edited by B. Schippers and W. Gams

October/November 1979, xvi + 686pp., £27.00/$62.50 0.12.624850.8

This book presents the proceedings of the fourth International Symposium of Soil-borne Plant Pathogens which was part of the third International Congress of Plant Pathology, held in Munich in August 1979. Much of the research currently being done on soil-borne plant pathogens is aimed at finding biological and integrated control of the diseases they cause, and it was this aspect of the subject which was emphasised throughout this meeting. Among the topics discussed were the dynamics and density of populations of soil-borne plant pathogens, the effects of changes in soil and crop management, side effects of pesticides on non-target organisms, interactions with other micro-organisms, and the biotic and abiotic factors of the soil ecosystem antagonising and suppressing pathogen populations and disease development.

Economic Microbiology Volume 3

Secondary Products of Metabolism

edited by A. H. Rose

October/November 1979, xvi + 590pp., £34.00/$78.50 0.12.596553.2

This third volume in the series is, like the other volumes, intended to provide an authoritative and lasting reference work on a separate topic in industrial microbiology, with particular emphasis on the chemical activities of micro-organisms. Volume 2 dealt with primary products of metabolism, and here the concern is with these secondary products that have economic importance. Following a general chapter on the commercial production of secondary products of micro-organisms, the authors discuss the basic microbiology and biochemistry of various groups of these compounds. More than half the book is devoted to antibiotics, for which there is an abundance of data, and the rest of the volume contains chapters on several other important groups of secondary products.

Microtubules

edited by R. Roberts and J. S. Hyams

October/November 1979, c600pp., £34.00/$71.50 0.12.590750.8

The last fifteen years have seen a vast increase in our knowledge of microtubules and their functions. The editors of this work feel that the first phase of microtubule research is almost at an end and that the next phase is slowly emerging. This book concentrates on those areas of research which are genuinely exciting now and are going to form the basis for future advances in this field. Important and key questions which will be answered in the next decade or so will depend for their answers on the core of information presented in this book. It covers such topics as the molecular structure of tubulin, the mechanism and control of its *in vivo* assembly, how microtubules function in the cell in motility and mitosis, and the precise functioning of nucleotides, ions and accessory proteins.

Modern Aspects of Microwave Spectroscopy

edited by G. W. Chantry

October 1979, xvi + 514pp., £38.00/$89.50 0.12.168150.5

This book serves a dual purpose. First, in bringing together experts from these and other branches of microwave spectroscopy, it should go a long way towards reducing the isolation in which recent research has been conducted and reported. To this end a comprehensive bibliography has been included at the end of each chapter. Secondly, each topic receives extensive critical discussion, which forms an up-to-date commentary on the progress in that area. These factors combine to make the book a valuable addition to the library of any firm, research institute, university or government department involved with microwave research.

East African Mammals Volume IIIB

An Atlas of Evolution in Africa

Jonathan Kingdon

November 1979, iv + 430pp., £55.00/$126.50 0.12.408343.9

Naturalists will have been waiting eagerly for this penultimate part to the third volume of Jonathan Kingdon's treatise: *East African Mammals*. His extraordinary drawings that reveal fine structural and anatomical details with such clarity, and the dedication and thoroughness with which the text of earlier volumes was invested have earned international acclaim. As a *tour de force* of artistic and scientific merit there can be few comparisons in its field. Part B of the third volume moves on from the carnivores of Part A to the large mammals for which Africa is so famous: the elephants, rhinos, the equids including the strange Painted Quagga, the pigs, the hippopotamuses, the camels, the chevrotains and giraffes. Broadly, it sets out to portray the role of these beautiful animals in tropical Africa's ecology and the evolutionary and functional significance of their curious forms and markings. The searching text is enhanced throughout, as in earlier volumes, by the author's exquisite drawings and sketches.

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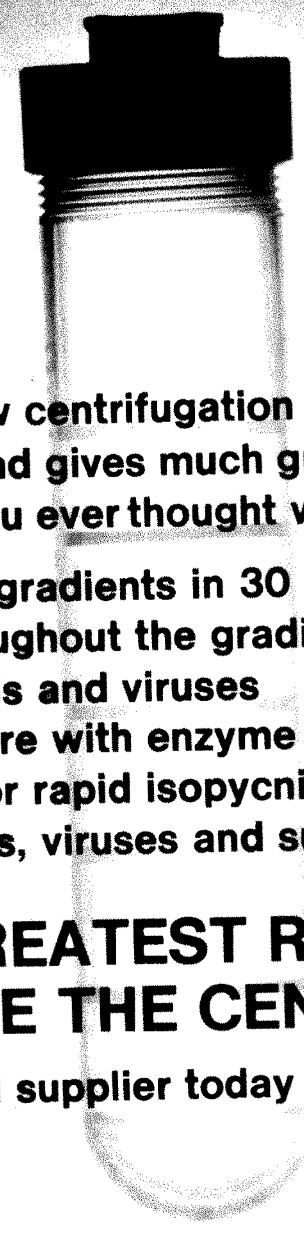

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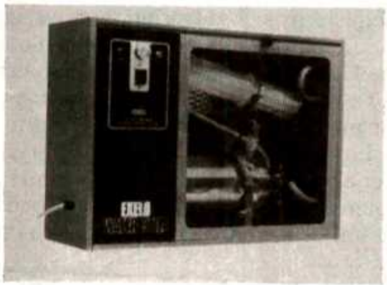

The Exelo enclosed water still

Desk-top computer/printer. Dynatech. A compact mini-computer/printer has been introduced by Dynatech Laboratories which can accept and perform computations on raw data from a wide range of instruments, such as Dynatech colony counters, which have a 4-bit BCD output. Designated the model 700, it is primarily designed for use with Dynatech model 980 and 880 macro/micro colony counters, but is also compatible with other similar counting systems. This 'desk-top' mini computer/printer can be removed from the primary instruments and used to handle a wide range of laboratory functions. Also, by removing a plug, the unit can be used to enter data manually. The range of equipment available includes a programmable calculator, a magnetic card programmable calculator and solid state software libraries. An 'alerting operator' programme ensures error-proof operation. At each stage of the computation the machine will inform the operator when more data are required to carry out the programme. The computer/printer module comprises a calculator which is locked onto the body of the printer. The thermal printer, which can provide alpha/numeric data and can plot curves and histograms, prints at a rate of 60 characters per s. Power requirements are 115 V, 60 Hz.

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Whole specimen preservators. VirTis. A range of preservator systems is available from VirTis, designed for use with their range of laboratory freeze dryers. Slow freeze-drying of whole specimens retains a physical appearance extremely close to that of the original. The absence of water in freeze-dried specimens allows safe storage and display at normal room temperature and humidity. Freeze drying is also the most labour-effective method of preparing a large number of specimens for teaching purposes. Refrigerated preservator chambers can be built large enough to house any animal, but for large specimens a more economical method is to place the vacuum chamber inside an existing storage freezer and a vacuum line run to the freeze dryer. A thermistor probe is then inserted into the deepest point of the animal to sense when it is fully frozen, and later when it is fully dried.

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Refractive index detector. Du Pont. The Du Pont refractive index detector is a stable drift-free instrument that is sensitive to any sample component whose refractive index is different from the mobile phase. Operation of the detector is based on Snell's law which measures the light beam deflection caused by the refractive index difference between the sample and the pure mobile phase. The unique flow cell housed in a Dewar flask eliminates the need for a liquid bath and minimises drift caused by temperature changes. Adjustable transparent plates are used to balance the light beam. Manual adjustment of the plates balances the light intensity of the two matched cadmium sulphide photocells.

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Automatic liquid nitrogen level controller. Edwards. Liquid nitrogen levels in process and storage vessels can be controlled automatically and reliably using the ALC7, a new, compact instrument from BOC Cryoproducts. The ALC7 uses two diode sensors to provide high and low level probes for pre-set level control, and a third probe to operate an audio low level alarm. For operator safety the ALC7 uses a stabilised 12 V, d.c. power supply unit which is connected to the mains. The controller operates a 12 V Cryoproducts Dewar pump or solenoid valve fitted to a suitable storage vessel, providing also a visual level indication by means of indicator lamps. A manual override button is also provided.

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One of the Carle Series-Sx gas chromatographs

Gas chromatograph. Carle. There is now a new facility for the range of gas chromatographs from Carle Instruments. The Series-Sx offers automatic specific analyses under control of the laboratory's data handling system or a microprocessor integrator with external timed event capability. Operation is based on automatic sample injection and column switching by motor-driven valves. The instrument has separate control channels for operations of up to four valves, or a combination of valves and other functions such as recorder on-off or solenoid operation. The Series-Sx is offered in all 20 application configurations currently available from Carle.

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These notes are based on information provided by the manufacturers. For further details circle the appropriate numbers on the Reader Enquiry Card bound inside the cover.

Dynamic balancing machine. B & K Laboratories have introduced a new universal balancing machine, the Type 3905, which is designed for use in conjunction with a new all electronic balancing machine console, the Type 2504. The 3905 is designed for 2-plane balancing of work pieces from 10 kg down to 0.5 kg, at either 1,500/1,800 or 3,00/3,600 r.p.m. A precision cardan shaft end-drive to the work piece permits positive and fast identification of the unbalanced position in both correction planes. The 3905/2504 combination permits a balance of quality grade G1 (ISO 1940) to be obtained and the unbalanced reduction ratio achievable is 90–95%.
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Digital cassette recorder. Memodyne has introduced a new computer controlled cassette recorder system called the M80. With a data capacity of 500 kilobytes, the M80 can transfer this data at programmed rates up to 19,200 baud. All potential errors are automatically retried and rewritten if the tape medium is at fault. All recorder controls and communication functions are interrupt driven and transparent to user programs. Two spare bus slots permit additional chips of dynamic RAM to be used for interfacing other peripherals such as keyboards, displays, complete terminals and printers.
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Array processor. CSPI. A 64-bit array processor, designed for scientific and engineering applications, has been announced by CSP. The new MAP-6400 interfaces to most popular 16- and 32-bit computers. All arithmetic operations are full floating point in a 64-bit hexadecimal format which provides over 16 decimal digits of precision. The major feature of the MAP-6400 is its unique combination of speed and precision at modest cost. The MAP-6400 has its own internal operating system and executive processor which performs all sequencing and control of tasks.
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X-Ray tube. IBM. A new kind of X-ray tube for the microscopic study of living cells has been invented by an IBM research scientist. The new tube produces short bursts of X rays that are enormously more intense than can be obtained from conventional tubes, or even from particle accelerators such as synchrotrons. In exposing X-ray sensitive materials, the new tube can produce an exposure in 100 ns. The tube is small, simple and inexpensive, and 'soft' X rays, with a wavelength of 10–100 Å, a wavelength range ideal for exposing X-ray resists. The tube achieves its remarkable output intensity by creating an extremely dense and hot plasma and concurrently sending through the plasma an electron beam with an intensity of 200 MA cm^{-2}.
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Catalogue of chemicals and supplies for radioassay. Packard. The new catalogue of chemicals and supplies lists the products which Packard has available for liquid scintillation and gamma counting. The 26-page catalogue now includes a "Sample Preparation Guide" to help the laboratory technician find the best sample preparation method and scintillation cocktail for 14 different groups of samples. The guide highlights some of the new scintillation cocktails introduced by Packard. The Chart and Table section provides reference tables that are handy for the laboratory, and includes decay tables, information on counting statistics and the properties of common radionuclides.
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Automatic blood oxygen dissociation analyser. TCS. The continuous oxygen equilibrium curve of blood can easily be obtained with the TCS Hemox-analyzer. Less than one drop of whole blood or haemolysate and less than 20 min of recording time are required to plot a complete oxygen dissociation curve, and thus obtain the P_{50} value of haemoglobin. The Hemox-analyzer provides an accurate and repeatable dissociation curve of normal and abnormal haemoglobins, including cord blood. No special preparation of the blood sample is required, as the blood is simply added to the buffer–serum solution before starting the recording.
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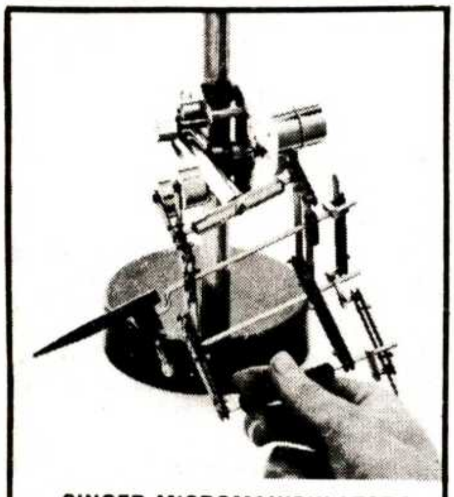

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Laboratory hotplate. Corning. A new generation of laboratory hotplates is announced by Corning. This range comprises the Model PC-35 (hotplate) Model PC-351 (hotplate with integral magnetic stirrer) and Model PC-353 (magnetic stirrer only). All three models have a unique design feature in the Pyroceram top plate, a tough glass ceramic. Pyroceram is pretreated for resistance to thermal shock, and offers considerable advantage over iron, aluminium and steel-topped competitive models, due to its resistance to corrosion, excellent uniform thermal conductivity for even heat-up, strength, and an easy to clean surface. Models PC-351 and PC-353 with stirring facilities are supplied with a corrosion-resistant PTFE-coated stirring bar. A powerful magnetic motor located under the top plate guides the stirrer bar with complete control at speeds variable up to 1,000 r.p.m. Surface area of all models is 225 cm^2 and temperature settings are variable up to 510°C. Heat-up is uniform over the whole top and maximum temperature can be achieved in 9 min.
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Eyeshield. Gallenkamp have introduced an eyeshield to be used as protection against UV light. The lenses, shade 3 BS 679, are tinted and screen out all wavelengths down to 200 nm. Made of nylon, the eyeshield has hinged sidepieces and earbows.
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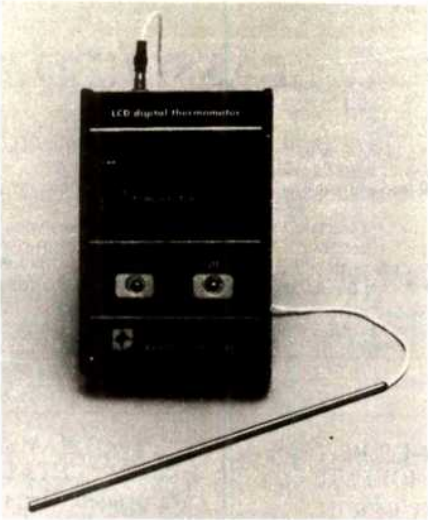

Pocket digital thermometer. Forma Scientific has introduced a new pocket digital thermometer designed for checking temperatures in ultra-low-temperature freezers, blook bank red cell and plasma freezers, laboratory refrigerators, environmental chambers, incubators and other laboratory equipment. Features of this new thermometer include: temperature range from −90.0°C to +60.0°C; digital readout to within 0.1°C; quick-response platinum probe; battery-saving liquid crystal display; quick disconnect 6 ft lead wire for use with multiple probes; finger touch on/off control.
Circle No. 102 on Reader Enquiry Card.

Biological safety cabinet. Nuaire. The Labgard class II, type 'B' biological safety cabinet has been introduced by Nuaire. The type 'B' cabinet has a vertical sliding sash and is designed to maintain an inward airflow of 100 LFPM at a work opening of 8 inches with an average downward vertical air velocity of 50 LFPM. The design requires separate exhausting of 70% of the air flowing through the work area and recirculates 30% through the HEPA filtration system. The cabinet is suitable, with separate exhaust system, for work with dilute preparations of chemical carcinogens.
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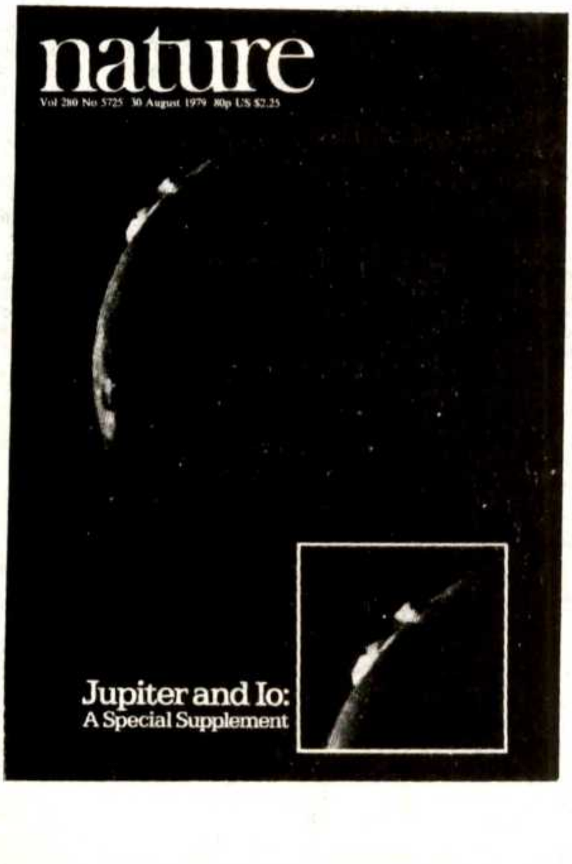
Asbestos-free insulation for laboratories. Lab Safety Supply. A new publication, entitled *High Temperature Materials for the Laboratory,* provides information on asbestos substitutes such as ceramic and high temperature synthetic fibres. It provides detailed physical and chemical properties of these materials and suggested laboratory uses. In addition, it discusses former uses for asbestos, its health hazards and current regulation. Also included is information about non-asbestos laboratory products made with these materials. These include gloves, tapes, ceramic centred wire gauze, hot pads, filter media and high temperature insulation. To obtain a free copy, write to: Lab Safety Supply Company, P.O. Box 1368, Janesville, WI 53545.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ratory, Madingley Road, Cambridge
0HE before 31 October. 3407(A)

UNIVERSITY OF BRISTOL
CHAIR IN VETERINARY MEDICINE

The University invites applications for appointment to the Chair in Veterinary Medicine which will become vacant on 1st August 1980, following the retirement of Professor C.S.G. Grunsell.

Candidates, who must have a veterinary qualification, are invited to submit applications, including the names of three referees, not later than 30th November 1979.

Further particulars of the appointment may be obtained from the Registrar and Secretary of the University, Senate House, Bristol BS8 1TH. 3291(A)

MEDICAL RESEARCH COUNCIL
National Institute for Medical Research
LABORATORY OF DEVELOPMENTAL BIOCHEMISTRY

Applications are invited from

POSTDOCTORAL SCIENTISTS

for a position to join a group working with Dr J.R. Tata in the area of regulation of gene expression during development. The post is particularly suitable for a scientist interested in applying techniques of recombinant DNA to questions concerning hormone action and genomic organisation and transcription.

The appointment will be for up to three years and will be in the present salary range £4,940 to £6,627 per annum plus £502 p.a. London Allowance; superannuation provision.

Applications, giving a full curriculum vitae, research experience and the names of two professional referees, should be sent to the Director, National Institute for Medical Research, London NW7 1AA before 15th November 1979 quoting reference DB.1. 3408(A)

CSIRO AUSTRALIA

Post doctoral Research Fellow
Centre for Animal Research and Development Bogor Indonesia

CSIRO has a broad charter for research into primary and secondary industry areas. The Organization has approximately 7,000 employees — 2,400 of who are research scientists — located in Divisions and Sections throughout Australia.

Field: Poultry Research

Duties: An experienced poultry research scientist is required to lead a research programme on Chicken Management and Nutrition at the Centre for Animal Research and Development in Indonesia. The aim is to develop appropriate management and feeding systems for both the large and small scale Indonesian poultry producer. The primary research effort will be directed to improving the efficiency of utilization of indigenous feedstuffs for chicken meat and eggs. The appointee will be expected to work closely with scientists working in an allied Duck Research Programme.

The centre has excellent facilities and staff for nutritional studies with chickens and there is a modern analytical laboratory and veterinary section to provide back-up service. Existing studies have focused on the evaluation of local feedstuffs and development of new feed resources. The new appointee will be expected to further the research in these areas, and to consider the general problems of management of small chicken flocks at the village level.

Qualifications: PhD and wide experience in the field of poultry research with an understanding of poultry nutritional experimentation. Experience in a tropical developing country an advantage. The training of Indonesian scientists will be an important responsibility and the successful applicant will be expected to show an interest and aptitude in this area.

Salary: Senior Research Scientists/Principal Research Scientist: $A20,198 — $A27,212 pa.

Tenure: Subject to negotiation, but a minimum of 3-4 years is envisaged. The appointee may be offered an indefinite appointment within CSIRO.

Conditions: Overseas conditions of service apply with respect to housing and allowances. As training of local staff is an integral part of the duties, it is essential that the successful applicant attain a degree of fluency in Indonesian language. To this end, a language course will be arranged.

Applications IN DUPLICATE, stating full personal and professional details, the names and addresses of at least two professional referees, and QUOTING REFERENCE NUMBER 1000/050 should reach:—

The Personnel Officer, Australian Scientific Liaison Office, Canberra House, 10-16 Maltravers Street, London WC2R 3EH, by 6th November 1979

Applications in U.S.A. and Canada should be sent to:— The Counsellor Scientific, Embassy of Australia, 1601 Massachusetts Avenue, N.W., Washington, D.C., 20036, U.S.A. 3413(A)

Scientist

required to work on

Motion Sickness

at the Institute of Naval Medicine, Alverstoke, Hants, with support administered by the MEDICAL RESEARCH COUNCIL in a 3 year project. The appointee will be of post-doctoral status preferably with some experience in physiology and/or pharmacology. Medical qualifications and/or experience of working with human subjects advantageous.

Salary according to qualifications, experience and age, in the range: £5,200 to £6,930.

Further particulars from the Assistant Secretary, Royal Naval Personnel Research Committee, Room 407, First Avenue House, 40-48 High Holborn, London WC1V 6HE. 3381(A)

MRC
Medical Research Council

TECHNICAL OFFICER (COMPUTER APPLICATIONS)

Applied Physics Division

MATHEMATICS SECTION

The successful candidate will join a team in the Mathematics Section whose work covers a range of research areas in addition to providing a mathematical service within CERL. He/she will work closely with Research Officers engaged on a number of problems for which numerical and computation solution plays a large and important role. In addition he/she would contribute to the mathematical and computational service provided by the Section.

Applicants should ideally possess an HNC or equivalent qualification and at least two years experience of computer programming. The duties of the post require ideally a good understanding of mathematics and numerical analysis, together with a knowledge of a range of computer languages and modern programming techniques.

The appointment will be made within a salary range of £5200-£7630.

The Laboratories are situated in a pleasant part of Surrey and offer attractive Conditions of Service, and facilities for the total of 800 Research and Support Staff engaged in a broad spectrum of research into the materials, technologies and plant performance problems of the Central Electricity Generating Board.

Application forms are obtainable from the Head of Personnel Development & Services, Central Electricity Research Laboratories, Kelvin Avenue, Leatherhead, Surrey KT22 7SE or telephone Leatherhead 74488 Ext. 363, quoting reference number **RL/37/N** Closing date is: **Friday 19th October 1979.**

CENTRAL ELECTRICITY RESEARCH LABORATORIES

3375(A)

ROYAL FREE HOSPITAL

KATHARINE DORMANDY HAEMOPHILIA CENTRE & HAEMOSTASIS UNIT

Pond Street, London NW3 2QG

POST-DOCTORAL BIOCHEMIST

A position is available now to join a team working on the interaction of blood coagulation factors. The work involves purification to homogeneity of human blood coagulation proteins VIII, IX and X from normal and haemophilic blood. The proteins will be specifically labelled so that binding to platelets and activation can be followed simultaneously by tracer techniques. The study is supported by the MRC and represents a new approach to the biochemical lesion in haemophilia.

The Haemostatis Unit is housed on two floors of the newly-built Clinical Sciences Block of the Royal Free Hospital School of Medicine. The appointment is for 3 years, salary 1A.

Further details may be obtained from Dr E.G.D. Tuddenham at the above ess, Tel. No. 01 794 0500, Ext. 3806, to n curriculum vitae and names of three es should be sent by those applying post. 3374(A)

THE HARVARD SCHOOL OF PUBLIC HEALTH

is seeking an outstanding scholar in the population sciences to fill the newly created

CLARENCE JAMES GAMBLE PROFESSORSHIP

In addition, the incumbent will be the Chairperson of the Department of Population Sciences. According to the terms of the gift, the incumbent should be a person highly qualified and involved in the development of programs which have relevance for human population control and/or the investigation of social, cultural, ethnic, religious, economic or other factors which influence the relationship between human populations and resources. The Search Committee is particularly anxious to fill the Chair with an individual having a broad outlook on the role of population sciences and problems of population control. Women and members of minority groups are especially invited to apply.

Qualified individuals are invited to apply by sending a current curriculum vitae and the names of five references to:

M. Zelen, Chairman, Gamble Search Committee, Department of Biostatistics, Harvard School of Public Health, 677 Huntington Avenue, Boston, MA 02115. Harvard University is an Equal Opportunity Employer with an Affirmative Action program. W409(A)

POSTDOCTORAL

to join small group working on regulation of transcription and translation during spermatogenesis. Experience in nucleic acid or protein chemistry useful. Contact: Dr N Hecht, Dept of Biology, Tufts University, Medford, Mass. 02155.

W410(A)

THE UNIVERSITY OF NEBRASKA-LINCOLN

BIOCHEMIST

Postdoctoral position available December 1 for research on the genetic control of cell differentiation. Our system utilizes 8 mutants affecting pigment production in cultured chick embryo pigment cells. Electrophoretic approaches will be used including PAGE, iso-electric focusing and 2-D separations. Previous experience in electrophoretic techniques required.

Appointment renewable annually with beginning salary $13.000. Send curriculum vitae and three letters of recommendation by November 1 to: Dr John Brumbaugh, School of Life Sciences, The University of Nebraska-Lincoln, Lincoln, Nebraska 68588.

Affirmative Action/Equal Opportunity Employer. W403(A)

UNIVERSITY OF SHEFFIEL

ELECTRON MICROSCOPY TECHNICIAN (Grade 5)

required for the

DEPARTMENT OF HUMAN BIOLOGY & ANATOMY

Applicants should have experience i the operation, and at least basi maintenance of transmission electro microscopes. Experience in biologic specimen preparation, while valuabl is not essential. Salary on scale £3,7 to £4,320 p.a.

Please send details of qualification experience and the names of tw referees to the Administrative Offic (Personnel), (Ref. S1362/N), or wri informally to Professor R. Bare Department of Human Biology an Anatomy, the University, Sheffiel S10 2TN. 3401(A)

CENTRAL PUBLIC HEALTH LABORATORY

NATIONAL COLLECTION OF TYPE CULTURES

MEDICAL LABORATORY SCIENTIFIC OFFICER

OR

SENIOR MEDICAL LABORATORY SCIENTIFIC OFFICER

Medical Laboratory Scientific Offic with minimum 4 years' experien post-AIMLS or equivalent or a Seni Medical Laboratory Scientific Offic (recently qualified FIMLS) is requir for the National Collection of Ty Cultures. Duties will inclu maintenance and testing of Collecti strains, examination of strains sent f identification, and opportunity assist in research programmes. A interest in bacterial classification a identification would be an advanta Experience will be gained with a wi range of bacteria.

Further details and applicati forms available from: The Person Officer, Central Public Heal Laboratory, Colindale Avenu London NW9 5HT. Tel: 01-205 704

Salary: M.L.S.O. scale £3,768 £5,391 plus £354 London Weighti Senior M.L.S.O. scale £5,016 to £6,5 plus £354 London Weighting. Closi date 31st October, 1979. 3348(A)

UNIVERSITY OF ST. ANDREWS

DEPARTMENT OF BIOCHEMISTRY & MICROBIOLOGY

Applications are invited for a post Graduate Scientific Assistant (Gra 1B) in the above Department, tena for two years from 1st January 19 The person appointed will work w Dr M G Burdon on the interaction $\&_2$ — macroglobulin with cultu fibroblasts from patients with cy fibrosis. Experience in tissue cult would be of great value.

Starting salary at appropriate po within range £3,775 to £4,333 (un review) plus FSSU/USS.

Applications with the names o referees should be sent by 31 Octo 1979 to Dr M G Burdon, Departm of Biochemistry & Microbiology, T University, St Andrews, Fife, fr whom further particulars may obtained. 3394(A)

THE UNIVERSITY OF SHEFFIELD

Postdoctoral Research Appointments in Theoretical Chemistry

Applications are invited from men and women for two post-doctoral positions supported by the SRC under a grant to Professor R. McWeeny and Dr B.T. Pickup.

Applicants should have some experience in the general area of molecular quantum mechanics and should be prepared to work on the application of propagator and/or density functional techniques to the calculation of molecular electronic structure and properties. Tenable immediately for up to two years. Initial salary up to £4,910 a year.

Applications, including curricula vitae and the names of referees, to Professor R. McWeeny, the University, Sheffield S10 2TN from whom further information is also available. Quote ref: R371/G.

3362(A)

UNIVERSITY OF BRISTOL

CHAIR IN VETERINARY SURGERY

he University invites applications for pointment to the Chair in Veterinary irgery which will become vacant on 1st nuary 1980, following the retirement of ofessor G. Arthur.

andidates, who must have a veterinary alification, are invited to submit plications, including the names of three erees, not later than 30th November 79.

rther particulars of the appointment ay be obtained from the Registrar and cretary of the Univesity, Senate House, istol BS8 1TH. 3290(A)

UNIVERSITY OF SURREY

EPARTMENT OF METALLURGY ND MATERIALS TECHNOLOGY

plications are invited for the post of dland Research Fellow tenable in the partment of Metallurgy and Materials chnology at the University of Surrey.

he research investigations will relate sely to the new product and technology grammes of Redland Ltd., a Company h world wide interests in building terials and related products. The Fellow carries out a number of subsidiary estigations requested by the Company's earch and Development Centre. The t affords an excellent opportunity for incumbent to familiarize himself with ustrial research and to make the nsition from academic to the industrial ironment.

pplicants should have a PhD or ivalent experience in some area of erial science, or solid state chemistry. ference may be given to candidates with rests or experience in silicate chemistry, land Cement and ceramics. In view of nature of the appointment, candidates ing the ability to formulate new erimental approaches to unresolved blems would be preferred. The Fellow ointed will undertake research in erials of interest in construction and ding and the main concern will be with perty structure relationships of interest eveloping new or improved materials.

he appointment would be made for two s and can be renewable.

emuneration will depend on age and rience and will be in the range £4,232 to 76 (under review), on scales related to University lecturer scales.

lications, from men and women, in the n of a curriculum vitae, with the names addresses of three referees, should be to: Professor J. E. Bailey, Department letallurgy and Materials Technology, ersity of Surrey, Guildford, Surrey 5XH. 3399(A)

The following Scientific vacancies exist in the Gwynedd Division of the Welsh Water Authority:—

TECHNICAL ASSISTANT (DATA) Reference 6SO17

Grade 4 (£4,887 – £5,235)

Applications are invited from persons either with a qualification in statistics and/or computer application or a qualification in chemistry and a special interest in statistics or data processing.

The post will be in the Divisional Scientists Department and will be based for several months at Caernarfon before the department moves to new offices at Bangor.

The work will involve data processing of treatment and pollution control information, both manually and by computer, statistical analysis and some mathematical modelling.

TECHNICIANS—2 Posts—Reference: 6SO37/6SO38

Grade 2 (£3,717 – £4,125)

Applications are invited for two posts within the Divisional Scientist Department. The work will involve routine sampling of natural waters (inland and tidal) and effluents and the investigation of pollution problems.

A qualification in chemistry or biology or a related subject would be an advantage. Previous experience would be an advantage but not essential. Candidates must hold a valid driving licence.

One post will be based at an Area Operations Office at Llandudno Junction (Ref: 6SO37) and the other post will be based at Dolgellau (Ref: 63038).

The Terms and Conditions of employment for all posts are as laid down by the National Joint Council for Water Service Staffs.

Application forms may be returned from the undernamed, the relevant reference numbers should be quoted in all cases.

Personnel Officer (Gwynedd),
Welsh Water Authority,
Bron Castell,
High Street,
BANGOR.

Tele: Bangor (0248) 52881 Extn: 51 or 52

Closing date for all posts: 19 October, 1979.

3405(A)

WELSH WATER AUTHORITY
AWDURDOD DWR CYMRU

UNIVERSITY OF DUNDEE

DEPARTMENT OF BIOCHEMISTRY

POST DOCTORAL RESEARCH FELLOW

Applications are invited for a post doctoral research post (MRC funded) to study the regulation of the synthesis of anaerobic enzymes in *Escherichia coli*. Ideally the successful candidate will have had some previous experience of the techniques of DNA sequencing and/or *in vitro* protein synthesis.

The appointment is tenable for one year initially, with the possibility of extension for a further two years at a starting salary of up to £5,193 (under review) depending upon experience.

Informal enquiries may be made to Dr B A Haddock, Department of Biochemistry, University of Dundee, Dundee DD1 4HN (telephone 0382 23181 Ext 551).

Applications including curriculum vitae and the names and addresses of two referees should be sent to The Secretary, The University, Dundee DD1 4HN quoting Reference EST/84/79J as soon as possible.

3355(A)

CENTRAL PUBLIC HEALTH LABORATORY

DIVISION OF ENTERIC PATHOGENS

MEDICAL LABORATORY SCIENTIFIC OFFICER

The post is in the phage-typing and drug resistance unit. The successful applicant will assist in the routine work of the unit but will be expected to participate in the research programme and would be encouraged to work for higher qualifications such as a higher degree or FIMLS by thesis as appropriate.

The Division is the National Reference Centre for *Salmonella, Shigella* and *Escherichia coli*. The World Health Organisation Collaborative Centre for Phage-typing and Drug Resistance of Enterobacteria is located in the Division.

Suitable qualifications would be a relevant science degree, HNC or AILMS with experience in Microbiology.

Applications to: Personnel Officer, Central Public Health Laboratory, Colindale Avenue, Colindale, NW9. Tel: 01 205 7041. 3347(A)

WORCESTER COLLEGE OF HIGHER EDUCATION

County Council of Hereford and Worcester

Appointment of Lecturer II in Human Physiology

Applications are invited from candidates with appropriate first degree and higher research degree to lecture in Human Physiology in the B.A. (Hons.) in Combined Studies and B.Ed.(Hons.) Programmes. The appointment will be made from 1st January 1980 or as soon thereafter as possible.

The successful candidate must have particular interest in the physiology of nutrition and exercise, and will be expected to play a leading role in the development of courses in Human and Social Biology.

The salary will be at the appropriate point on the Lecturer Grade II Scale, £4,542 to £7,221.

Application forms and further particulars may be obtained from The Principal, Worcester College of Higher Education, Henwick Grove, Worcester, to whom completed application form should be returned as soon as possible.

3391(A)

Cardiovascular Pharmcologists

Greenford, Middx.

With the appointment of Dr. D. W. Straughan, B.Sc., Ph.D., M.B., B.S., L.R.C.P., M.R.C.S., as Director of Pharmacology, the Greenford Division of Glaxo Group Research Ltd. is seeking two young and enthusiastic pharmacologists to join the Cardiovascular Section of the Pharmacology Department.

While there is some flexibility regarding the precise responsibilities attaching to the two posts, it is envisaged that one position will be filled by a recently qualified graduate with a good honours degree who now wishes to develop an interest in cardiovascular research. The second post will be suitable for a person with previous research experience relevant to the development and evaluation of new compounds of potential value in the treatment of cardiovascular diseases. A sound knowledge of current concepts regarding the actions of drugs on receptors and the implications for drug design is considered essential.

We offer competitive salaries and excellent conditions of employment, including pension and bonus schemes, a subsidised cafeteria, and a sports and social club.

Please write, or telephone, for an application form to:— Mrs. C. A. Carter, Personnel Officer, Glaxo Group Research Limited, Greenford Road, Greenford, Middlesex. Tel: 01-422 3434, ext. 11. Ref. no. 282. 3412(A)

Glaxo Group Research Ltd.

SCOTTISH MARINE BIOLOGICAL ASSOCIATION

DUNSTAFFNAGE MARINE RESEARCH LABORATORY

nr. Oban, Argyll.

Applications are invited for the post of

DEPUTY DIRECTOR

at the Dunstaffnage Marine Research Laboratory.

The Dunstaffnage Laboratory is multidisciplinary, undertaking basic, strategic and applied research in coastal waters and the deep sea. Some postgraduate teaching is undertaken in conjunction with Stirling University. The staff number about 100 and in addition there are about 12 postgraduate students. The Laboratory, which is independent, is funded by a grant from the Natural Environment Research Council and by contract work, and has an annual budget of about £950,000.

Candidates will be expected to have an established reputation in marine science and an aptitude for laboratory administration. The post is graded at Senior Principal Scientific Officer level, and the appointee will be required to take part in the management of the research and administration of the Laboratory and deputise for the Director in his absence.

Application forms and further details may be obtained from the Administrative Officer, Dunstaffnage Marine Research Laboratory, P.O. Box 3, Oban, Argyll PA34 4AD. Closing date for receipt of application forms: 26 October, 1979.

3402(A)

UNIVERSITY OF NEWCASTLE UPON TYNE

BIOCHEMISTRY RESEARCH DEMONSTRATORSHIP

Applications are invited for the post of

DEMONSTRATOR

in the Department of Biochemistry from candidates who possess, or have submitted for, a PhD in Biochemistry, for a period of up to three years, tenable from 1st January, 1980. Research projects currently in progress include membrane transport of nucleic acid precursors (Professor K. Burton), acquisition and modification of enzyme conformation, and immunochemistry of monocyte surface (Professor R.H. Paine), DNA repair and genetic recombination (Dr P.T. Emmerson), triglyceride and lipoprotein metabolism (Dr M.P. Rogers), enzymology of resistance to antibiotics (Dr R. Virden), lactose transport and bioenergetics (Dr I.C. West) and hormonal control of enzyme activity (Dr S.J. Yeaman). Duties will also include teaching (approximately 90 hours per year) by lectures, tutorials and practical classes.

Salary will be at an appropriate point on the Grade 1B (Bar) scale £3,775 to £5,488 per annum according to age, qualifications and experience. Membership of the appropriate University superannuation scheme will be required.

Further particulars may be obtained from the Senior Assistant Registrar (F.P.) The University, 6 Kensington Terrace, Newcastle upon Tyne, NE1 7RU, with whom applications (2 copies), giving the names and addresses of three referees, should be lodged as soon as possible.

3376(A)

UNIVERSITY OF SOUTHAMPTON

FACULTY OF MEDICINE

DIAGNOSTIC RESEARCH UNIT

Applications are invited for the following posts:—

TWO EXPERIMENTAL OFFICERS

Funds are available for the above posts in the recently established Diagnostic Research Unit which is closely integrated with the Department of Medicine (Professor Ralph Wright). The objective is to develop original serological procedures for widespread diagnostic application in hospitals and biochemical and immunological laboratories. The appointees will be expected to use initiative but would receive general direction from the Senior Experimental Officer in the Unit. The work will require a broad knowledge of biochemical and immunological techniques. Preference will be given to post-doctoral biochemists/immunologists.

The appointments will be for one year initially but may be renewed on an annual basis.

Salaries within the scale £3,775 to £6,355 p.a.

For further details contact Dr F.G. Bull on Southampton 777222 ext. 3910 or 4299.

Applications giving details of qualifications and experience and the names of two referees should be sent to Mrs P. Vaughan-Smith, Staffing Section, The University, Southampton, SO9 5NH, as soon as possible. Please quote ref: 1097/A/N. 3356(A)

STANFORD UNIVERSITY

GEOCHEMIST —PETROLOGIST

The Department of Geology in the Scho of Earth Sciences invites applications fo faculty position beginning Septemb 1980. Areas of interest include (but are n limited to) geochemical processes in t crust and mantle of the earth includi isotope geochemistry, thermodynami kinetics, element distribution and pha equilibria studies. Teaching responsibilit might include graduate and undergradu courses in geochemistry and igneo petrology. Level of appointment is ope but we are particularly interested in creative young person, with a we established record in research.

Applications should include (1) a resu of education, professional experience, a published research; (2) a brief statement plans for future research, and (3) the nam of 4 referees. Send to: Prof. J.G. Lio Department of Geology, Stanfo University, Stanford, CA 94305, December 1st, 1979. Stanford University an equal-opportunity/affirmative-acti employer. W329(A)

PAPUA NEW GUINEA INSTITUTE OF MEDICAL RESEARCH

Position Vacant

RESEARCH FELLOW IN IMMUNOLOGY

The Institute is a statutory body within Ministry of Health of the Government Papua New Guinea. It is involved in a w range of fundamental and applied resea related to the health of Papua N Guineans.

Location: Main complex situated Goroka, in the Eastern Highla Province, with a branch at Madang, an number of laboratories and field stati throughout the country.

Duties: To undertake research i immunological aspects of infecti diseases prevalent in Papua New Guine particular to participate in a mu disciplinary study of acute respirat infections. Other members of the resea team include an epidemiologist, a vi logist, a bacteriologist, a nutritionist an paediatrician. The successful applicant be expected to develop assays for detection of bcterial antigenaemia, an collaborate in vaccine trials, in additio other research activities arising out of applicant's own particular interests.

Qualifications: MD or PhD immunology or bacteriology, equivalent. Applicants with a Ma degree will be considered if their experi is particularly appropriate to the requ ments of the position.

Conditions of Service: Similar University of Papua New Guinea. Suit accommodation will be provided by Institute. For a contract appointme 3-year contract will be offered; recruit fares and expenses and leave fares e eighteen months will be paid.

Salary: Overseas salary payable w applicable (K10165 to K13725 plus annual gratuity of 24% for the first t years). (1K (kina) = 1.40 US$). Fo experienced senior applicant appoint at the level of Senior Research Fe (K15575 plus gratuity) would considered.

Inquiries and applications: Applicat should include a curriculum vitae, an names and addresses of at least referees, and be addressed to:

The Secretary, PNG Institut
Medical Research, P.O. Box
Goroka. E.H.P. Papua New Gu

W406(

LANCHESTER POLYTECHNIC
Coventry
DEPARTMENT OF BIOLOGICAL SCIENCES

SENIOR TECHNICIAN
(Animal Physiology)
£4,080 to £5,067

o be responsible for the animal science boratories and for servicing laboratory asses and research. Applicants should ave qualifications at degree, HNC or quivalent level and have relevant iboratory experience, including hysiological instrumentation, or 5 years levant experience.

NSTRUMENT TECHNICIAN
£3,585 to £4,533 or
£4,080 to £5,067

o assist in the maintenance and velopment of equipment, including a icrocomputer and its software. Training ill be provided as necessary. Applicants ould have professional or amateur perience in electronics and be qualified ith GCE 'A' level, ONC, TEC or City and uilds in either science or electronic actice; further qualifications and/or perience (5 years) will be required for an pointment at the higher grade.

Application Forms and job ecifications from Personnel Officer, nchester Polytechnic, Priory Street, ventry, CV1 5FB, returnable by Friday, th October, 1979. 3393(A)

IMPERIAL COLLEGE
DEPARTMENT OF CHEMISTRY

GRADUATE RESEARCH ASSISTANT (MRC funded)

required for up to 5 years to work on zymology, DNA replication and protein thesis. Salary in range £4,515 to £6,228 lusive.

Applications, plus c.v. and names of two erees to Professor A. R. Fersht, partment of Chemistry, Imperial llege of Science and Technology, ndon SW7 2AY. 3411(A)

THE UNIVERSITY OF LEEDS
DEPARTMENT OF BIOCHEMISTRY

plications are invited for a post of

RESEARCH FELLOW

the Department of Biochemistry. The ject, under the supervision of Dr S.B. own and supported by the Nuffield undation, will involve studies on the chanisms of oxidative degradation of m and chlorophyll derivatives. The post be suitable either for an organic mist interested in acquiring experience a biochemical environment or, rnatively, for a biochemist with some npetence in organic chemical hniques. Previous experience with apyrroles would be an advantage but is essential. A PhD degree is required. appointment will be made for a fixed od of three years and it is hoped that the cessful candidate will be able to take up post as soon as possible.

tarting salary in the range £4,333 to 488 on the 1A scale for Research and alogous Staff (£4,333 to £7,521) (under ew) according to age, qualifications experience.

pplication forms and further iculars may be obtained from the istrar, The University, Leeds LS2 9JT, ting reference number 83/25. Closing for applications 19 October 1979.
3319(A)

Research Associate Post in the Theory of Continuum States of Atoms and Molecules

The Computational Science Group of the Daresbury Laboratory is currently engaged in a research project on the continuum states of atoms and molecules. The work is being carried out in collaboration with a number of University groups.

A post attached to this project is offered for three years as a Research Associate; it is superannuable and in the salary range £4545 to £7712 per annum depending on age, qualifications and experience. Candidates will be expected to have a good knowledge of current theory of atomic and molecular processes and to have had relevant experience in the implementation of these theoretical models on a computer. The laboratory has powerful computing facilities available including IBM 370/165 and CRAY-1 computers on-site, and a link to two IBM 360/195 computers at the SRC Rutherford laboratory.

Applicants, of either sex, should preferably have or expect to obtain a PhD in Physics/Chemistry and should write (or telephone Warrington 65000, Ext. 407) for an application form quoting reference DL/705/CB to:—

The Personnel Officer
DARESBURY LABORATORY
Science Research Council
Daresbury, Warrington WA4 4AD

Closing date — November 16th 1979. 3387(A)

POSTDOCTORAL RESEARCH FELLOW IN GEOPHYSICS

The Division of Geological and Planetary Sciences at the California Institute of Technology expects to offer a Research Fellow appointment in the area of solid-state high pressure geophysics and/or descriptions of the high pressure phenomena associated with impact cratering on planetary surfaces.

Research fellowships in geology, general geophysics, geochemistry, geobiology, and planetary science are also available from time to time. Interested persons are asked to contact Dr Barclay Kamb, Division of Geological and Planetary Sciences, California Institute of Technology, Pasadena, California 91125. An Equal Opportunity/Affirmative Action Employer. W411(A)

ST. GEORGE'S HOSPITAL MEDICAL SCHOOL
(University of London)

RESEARCH TECHNICIAN

required for 3 year research programme in the Child Health Department. Duties involve programmes in infant nutrition, biochemical genetics, endocrinology and lipid metabolism in children. H.N.C. or degree in biochemistry or related subject required.

Emoluments range £3,700 to £4,584 plus London allowance £275 p.a. (under review). Enquiries to Dr N. McIntosh (01-672-1255 ext 4170).

Application forms from Establishment Officer, St. George's Hospital Medical School, Cranmer Terrace, London, SW17 0RE. 3379(A)

RICE UNIVERSITY
Announces the opening of a

CENTER FOR SPACE PHYSICS

Scientists with experience in theoretical or experimental space physics are invited to apply for continuing, full-time research staff positions at the level of Assistant, Associate, or Senior Research Scientist. (These three levels are roughly parallel to the three academic faculty ranks, except that they do not carry tenure and must be fully funded from external research grants and contracts.)

It is anticipated that the Center will promote several subdisciplines of Space Physics research, including computer simulation of magnetosphere studies. The specific area of emphasis will be determined by the individual interests of the research staff and by the nature of the grant/contract support that they are able to generate.

Applications and resumes should be addressed to Prof. A.J. Dessler (Chairman), Dept. of Space Physics and Astronomy, Rice University, Houston, TX 77001. The University is an equal opportunity/affirmative action employer.
W415(A)

SUPRA REGIONAL PROTEIN REFERENCE UNIT, CARDIFF

Applications are invited for the post of

RESEARCH ASSISTANT

to work on the development and assessment of immune complex assays in clinical chemistry. This project is supported by the Welsh Office scheme for the development of Health and Social research. The post will be for one year in the first instance, but renewal for the second year may be possible.

Candidates should have a degree in Biochemistry, Biological Sciences or equivalent and salary scales will be according to qualification and experience, but will be on the Whitley Scale for probationary grade hospital Biochemists.

Further details of the project and the post can be obtained from:
Dr. R. Fifield, Protein Reference Unit,
Cardiff Royal Infirmary, Tel: 0222 492233 Ext. 559

Application forms are obtainable from:
Assistant Unit Administrator, Cardiff Royal Infirmary,
Newport Road, Cardiff. Tel: 0222 492233.
Closing date: 22nd October, 1979 3373(A)

Universiteit van Amsterdam

The Department of Physics and Astronomy
of the University of Amsterdam
has an opening for the position of

lector* (m/f) in theoretical physics

This is a new position created to promote and stimulate co-operation in theoretical and experimental high energy physics. The appointed lector shall be a joint member of the Theoretical Institute and the High Energy Institute of the University. The latter is a participant in the National Institute for Nuclear and High Energy Physics (NIKHEF).

The field of research of the Theoretical Institute includes the theory of elementary particles, statistical mechanics, relativistic kinetic gas theory, and intermolecular interactions. The research of the High Energy Institute is in the area of bubble chamber experiments and counter experiments that are carried out with accelerators, in particular those at CERN, Geneva.

The functions of the lector shall be the following. The performance, stimulation and guidance of research in theoretical physics with emphasis on the forging of a strong bond between the two institutes. The giving of lectures in theoretical physics, in particular high energy physics. The guidance of graduate students in theoretical and high energy experimental physics.

People interested in this position are asked to submit a written application before December 1, 1979 including a curriculum vitae, a list of publications, and the names and addresses of a few references who can provide information concerning scientific and personal qualifications.

Suggestions of names of candidates for this function should be submitted before November 1, 1979.

Suggestions and applications should be sent to the chairman of the appointment committee,

Dr. T. de Jong,
Bureau Subfaculteit Natuur- en Sterrenkunde,
Nieuwe Achtergracht 170,
1018 WV Amsterdam, The Netherlands.

* In the Dutch academic system, the highest ranking faculty positions are professor and lector.

W404(A)

Chelsea Hospital For Women

Dovehouse Street, London SW3

is a small friendly 80 bedded gynaecological unit situated in a pleasant part of Chelsea, close to excellent shopping facilities and within easy reach of South Kensington tube station and on several bus routes.

Senior Biochemist

in the Department of Endocrinology. The department provides a hormone assay service to the hospital and carries out research work in the field of reproductive endocrinology. Experience in these areas would be an advantage.
Whitley Council terms and conditions of service. (Salary Scale £6,228 to £7,803, further increase from 1.1.80).

Application forms available from and returnable to the Administrative Assistant, 339 Goldhawk Road, London W.6. Tel: 01-748 4666 ext. 204.

3349(A)

UNIVERSITY OF LEICESTER

DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS

POSTDOCTORAL RESEARCH ASSOCIATE

Applications are invited for a postdoctoral research associateship to work on the characteristics and regulation of catecholamine receptors in the central nervous system. Experience in receptor labelling techniques and cyclic nucleotide metabolism would be advantageous but not essential. Applicants should be recent PhD graduates or have up to three years postdoctoral experience. The post is for two years and the salary will be in the Range 1A (£4,333 to £7,521) depending upon age and experience.

Applications giving details of qualifications, research experience and the names of two academic referees should be sent to Dr S.R. Nahorski, Department of Pharmacology and Therapeutics, School of Medicine, Medical Sciences Building, University of Leicester, University Road, Leicester. LE1 7RH (Tel: 0533 551234, Ext. 361). 3358(A)

INTERNATIONAL CENTER FOR TROPICAL AGRICULTURE AGRICULTURAL SCIENCES WRITER/EDITOR

The International Center for Tropical Agriculture (CIAT) generates and transfers technology for improved production of certain basic food crops. CIAT is seeking an agricultural science writer/technical editor to work with its research staff in developing articles and publications on its work. Audiences include agricultural scientists, national planners and policy makers in developing countries and various groups in developed countries working to improve food production in the Tropics. Some technical editing is also required.

Candidates must have proved ability to supervise editorial personnel. A minimum of three years of experience in agricultural science writing and editing is desired; message production experience in other media would be helpful. An MS or PhD in some agricultural field is preferred and fluency in Spanish is desirable. The position is at CIAT headquarters, Cali, Colombia.

Send letters of application, with curriculum vitae and three references, to Dr. Alexander Grobman, Director for International Cooperation, Centro Internacional de Agricultura Tropical, Apartado Aéreo 6713, Cali, Colombia. Closing date is November 15, 1979.

W408(A)

UNIVERSITY OF LONDON

INSTITUTE OF NEUROLOGY

Department of Neurochemistry

Post-doctoral Molecular Biologis Biochemist required for a research tea investigating the molecular basis of ment retardation. Experience in nuclear aci and protein synthesis essential. Tl position is tenable for up to 5 yea commencing January 1980. Starting sala will be up to £5,199, plus £740 Lond allowance, on range 1A, depending on a and experience.

Apply with c.v. and names of thr referees to Dr. L. Lim, Institute Neurology, 33 John's Mews, Lond WC1N 2NS (Tel: 01-242 4063). 3397(A)

UNIVERSITY OF EAST ANGLIA, NORWICI

SCHOOL OF CHEMICAL SCIENCES

Electron Energy Loss Spectroscopy of Adsorbed Molecules

Applications are invited for an

SRC POSTDOCTORAL RESEARCH

appointment to work with this new hig sensitive technique of vibratio spectroscopy in order to study the struct of chemisorbed species on single-cry planes of metals. Equipment for research will shortly be installed and combine electron energy loss measu ments with LEED and Au spectroscopy. Experience in application of electron spectrosco techniques in surface chemical phy would be of advantage.

The project is a joint one between E Anglia and Queen Mary College, Lon (Professor J. Pritchard) but the succes applicant will be based at Norwich. post is tenable for a period close to 3 ye depending on the initial salary which wi near point 3 of the RA1A scale (£4,776

Applicants should write to Professor Sheppard, F.R.S., or Dr M.A. Chest School of Chemical Sciences, Universit East Anglia, Norwich NR4 7TJ, enclos a curriculum vitae and the names addresses of two referees.

Director of Biology

G. D. Searle is a leading international pharmaceutical company with its main U.K. headquarters and research facilities at High Wycombe in Buckinghamshire.

Applications are invited for the position of Director of Biology from persons with qualifications and research experience in a Biological Science, preferably Pharmacology. This position reports to the Director of Research.

Responsibilities include directing the efforts of a number of groups of biological scientists working principally in the area of infectious diseases, immunology and tumour biology. In addition to drug testing programmes, studies on the mechanism of action of therapeutic agents and on the basic biology of the diseases are undertaken. The successful applicant must therefore have a broad view of biology and medicine, whilst being able to direct in depth studies in particular areas. The responsibilities also include management of the animal facilities, so direct experience is essential. Experience in supervision and administration of other licencees will be advantageous.

The groups within the Research Department are organised to encourage an interdisciplinary approach to most studies and past experience of this aspect within the Pharmaceutical Industry will be a strong recommendation. In addition evidence of managerial experience will be required.

This is a senior position, carrying a place on the Management Group of the Research Department, and the salary, which is negotiable, will reflect this seniority.

The benefits accompanying this post include: company car, relocation assistance, life insurance, contributory pension fund, private medical scheme, and sports and social club.

Applications should be addressed to Mr G. Jenkins, Personnel Manager, Searle Research & Development, G. D. Searle & Co. Ltd., Lane End Road, High Wycombe, Bucks (Tel. High Wycombe 21124, ext. 3374).

SEARLE

3396(A)

UNIVERSITY OF OXFORD

UNIVERSITY LECTURERSHIP/ CURATORSHIP IN ENTOMOLOGY

plications are invited for the above post. pend according to age on the scale ,333 to £9,581 (interim scale effective m 1.10.79). The successful candidate y be offered a tutorial fellowship by dy Margaret Hall.

Details may be obtained from the acre Professor of Zoology, Department Zoology, South Parks Road, Oxford 1 3PS, to whom completed applications ne typed copies, two from overseas didates) should be sent by 1 December 9. 3370(A)

UY'S HEALTH DISTRICT

BASIC GRADE BIOCHEMIST

389 pa. inc. rising on an incremental e to £5,289 pa. inc.

,818 inc. pa. rising to £5,670 inc. pa. as n January 1st 1980)

e have an important research vacancy a well qualified Biochemist. The arch carried out will be into the hemical consequences of renal failure. he post will be for two years in the first ance.

taff facilities at Guy's include a staff , swimming pool, squash courts, idised restaurants and excellent public sport links at London Bridge.

or further details and an application please contact the Personnel Dept., 's Hospital, St. Thomas Street, don SE1 9ET or phone 01-407 7600 Ext .

losing date for the receipt of completed ications 19th October 1979.

ambeth Southwark and Lewisham Health Authority (T). 3367(A)

ECOLOGY AND ANIMAL BEHAVIOUR

The Biology Department of McGill University offers two positions with a diverse and active group of terrestrial and aquatic ecologists at the level of assistant professor (both tenure stream), beginning in September 1980.

Send a curriculum vitae, a statement of research and teaching interests, and the names, addresses and telephone numbers of three potential referees to Dr. F. H. Rigler, Chairman, Department of Biology, McGill University, 1205 Avenue Docteur Penfield, Montreal, Quebec, Canada, H3A 1B1. Competition closes on December 15, 1979. W416(A)

UNIVERSITY OF CAMBRIDGE

RESEARCH ASSISTANT (Physics/Electronic Engineering)

A physicist or electronic engineer with graduate or equivalent qualification is required to join a team engaged in the development and application of ion-probe mass spectrometry. Experience with computer-based measuring systems is highly desirable and some familiarity with ion or electron optics and ultra-high vacuum equipment would be an advantage. The successful applicant may expect to gain experience of a wide range of experimental techniques.

The post is tenable until Feb 1982 with a starting salary in the range £4,055 to £4,622 per annum, depending on age and experience.

Applications, stating qualifications & experience, should be sent to Dr J.V.P. Long, N.E.R.C. Ion-Probe Unit, c/o Department of Geodesy & Geophysics, Madingley Rise, Cambridge, from whom further information may be obtained.

3363(A)

NEW ZEALAND

CENTRAL INSTITUTE OF TECHNOLOGY

SCHOOL OF PHARMACY

The Council of the Central Institute of Technology wishes to make appointments in the following subject areas and invites applications from suitably qualified persons:

(1) *Pharmaceutical Chemistry:* A person suitably experienced in the field of medicinal chemistry. Experience in teaching would be desirable but not essential. Applicants with qualifications and interests in closely related areas will be considered.

(2) *Pharmaceutics:* A person experienced in formulation technology. A particular interest in microbiological aspects would be advantageous but not essential.

The above positions would suit graduates interested in taking responsibility for specific sections of the Diploma course which is of three years duration and leads to the basic registrable qualification for New Zealand pharmacists.

Assistance with fares and transfer of baggage may be available to the successful applicant.

It is anticipated that initial appointments will be made to one of the following scales, depending upon qualifications and experience:

Scale 2: NZ$10,928 to NZ$14,126

Scale 3: NZ$13,229 to NZ$15,401

For a particularly well qualified and experienced applicant:

Scale 4: NZ$14,989 to NZ$16,549

Salaries are at present under review and will rise when the review is completed.

For further information and application forms write to either:

The Chief Migration Officer
New Zealand House
Haymarket
LONDON SW1Y 4TQ

or

The New Zealand Embassy
37 Observatory Circle
NW
WASHINGTON, DC
20008

Applicants writing to London should quote vacancy number Imm 2/323/5 CIT/2 : to Washington CIT/2. 3368(A)

Earth Scientists

Geothermal Research

We have an unusual and interesting opportunity for someone to take responsibility for a project in Geothermal Research. Based at our Geophysics Research Branch in London, you will construct, assess and use a computer model for the calculation of the thermal history of sedimentary basins with particular reference to organic diagenesis. This key project provides the chance for a strongly innovating role.

To be able to contribute to this project, you will probably have 3-4 years' post Ph.D./M.Sc. experience in the earth sciences. A strong numerical background and the ability to use the computer in this work, including programme writing, are important. Essential qualities are the ability to initiate new ideas and the drive to develop and use them and present them to others.

Our current salary levels, together with non-contributory pension scheme, subsidised lunches and excellent sports and social facilities, now represent outstanding conditions of employment.

Please write, giving details of age, qualifications and experience, quoting reference U.716 to: Sue Bartholomeou, Central Recruitment, The British Petroleum Company Limited, Britannic House, Moor Lane, London EC2Y 9BU.

BP

3404(A)

ST. GEORGE'S HOSPITAL MEDICAL SCHOOL

(University of London)

TECHNICIAN 3

(Biochemistry)

required to assist with an MRC sponsored investigation ending 30.9.82 into cell surface heparan sulphate and its possible involvement in growth control. Emoluments range £3,122 to £3,492. London allowance £275 (under review).

Application forms and further particulars from the Establishment Officer, St. George's Hospital Medical School, Cranmer Terrace, London, SW17 0RE. Closing date 25 October 1979

3377(A)

NATIONAL INSTITUTE FOR RESEARCH IN DAIRYING

(University of Reading)

CHEMISTRY DEPARTMENT

A graduate is required to work for three years on the effect of heat on the proteins and enzymes of human milk. The work involves undertaking a detailed study of the kinetics of their heat denaturation and relating their results to the effect of pasteurisation treatments on human milk.

Qualifications: First or Upper Second Class Honours degree in biochemistry or food science. An opportunity exists for a suitable candidate to register for a higher degree.

Appointment, for a limited period of only three years, will be as Scientific Officer; salary scale £3,289 to £5,085 (£3,591 to £5,486 from 1.1.80). Non-contributory superannuation.

Application forms obtainable from the Secretary, NIRD, Shinfield, Reading RG2 9AT. Quote reference: 79/32. 3350(A)

INSTITUTE OF CANCER RESEARCH: Post Doctoral Scientist required for a three year Fellowship in the Department of Cytogenetics and Immunogenetics, at Fulham Road, London, SW3, to work with human premalignant and malignant cells *in vitro*; the study will involve the genetics of cytogenetically marked cell populations maintained and manipulated in tissue culture and xenograft systems. Experience in tissue culture and cytogenetics essential. Salary in scale £4,261 to £6,627 p.a. (MRC Scientific Non-Clinical) plus London Allowance of £502 p.a. Applications in duplicate with the names of two referees to the Secretary, Institute of Cancer Research, 34 Sumner Place, London, SW7 3NU, quoting ref. 300/G/7 3382(A)

IMPERIAL COLLEGE

(University of London)

LECTURESHIP IN CELL BIOLOGY

The position is in the Department of Biochemistry but the person appointed will contribute to the teaching of cell biology in the division of life sciences. Candidates must have experience in electron microscopy. The department has strong interests in neurobiology, in which this lecturer will be expected to participate. Excellent facilities are available for transmission, scanning, stem/electron probe and freeze-fracture electron microscopy and for autoradiography.

Salary will be at the appropriate point in the lecturer scale £5,073 to £9,732 inclusive of London Allowance (to be reviewed).

It is intended to make this appointment at some time during the academic year 1979-80.

In the first instance, send a request for further particulars of this post to Professor E. A. Barnard, Department of Biochemistry, Imperial College of Science and Technology, London SW7 2AZ.

3409(A)

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF BIOCHEMISTRY

RESEARCH ASSISTANT

Applications are invited from graduates in Biochemistry or related subjects to work on a project investigating the role of intracellular proteinases in the initiation of protein catabolism. The project is funded by the SRC and is under the supervision of Dr R.J. Beynon.

The appointment will be made as soon as possible, at an initial salary of £3,775 per annum.

Applications, together with the names of three referees, should be received not later than 25th October, 1979, by The Registrar, The University, P.O. Box 147, Liverpool, L69 3BX, from whom further particulars may be obtained. 3352(A)

OXFORD UNIVERSITY

Sir William Dunn School of Pathology

PLASMA MEMBRANE BIOCHEMIST

Applications are invited for a post-doctoral research position, funded by a grant from the Medical Research Council. The research concerns the characterisation of macrophage antigens detected by monoclonal antibodies. Experience in membrane biochemistry and in immunochemistry is desirable. The appointment, starting as soon as possible after 1st October 1980, is likely to be for 3 years. The salary will be at an appropriate point on the University 1A scale. £4,333 to £7,145.

Applications, including *curriculum vitae* and the names and addresses of 2 referees, should be sent to Dr S. Gordon, Sir William Dunn School of Pathology, South Parks Road, Oxford OX1 3RE. 3369(A)

CITY OF LONDON POLYTECHNIC

GEOGRAPHY SECTION

LABORATORY TECHNICIAN

(Grade 4)

Applications are invited from suital qualified persons for the above vacan The person appointed will be required take responsibility for the day to c organisation of the Physical Geograp Laboratories including the provision materials for undergraduate classwork.

The Geography Section is located in C Castle Street, E1 within easy reach Fenchurch Street and Liverpool Str stations and Aldgate East Undergrou station.

Applicants must have ONC/HNC City and Guilds in Laboratory techniq or other appropriate qualifications.

The salary will be within the range £3, to £4,233 including £525 Lond Allowance (salary presently under revie Starting point is dependent on a qualifications and experience.

Good conditions of service inclu subsidised dining facilities, contribut pension scheme, 21 days annual lea (rising to 26) plus *an additional day off ea calendar month*.

Further details and an application fo can be obtained by writing (quoti references 79/116) to the Staff Recor Officer, City of London Polytechnic, Hounsditch, London EC3A 7BU.

3378(A)

UNIVERSITY OF BATH

SCHOOL OF PHARMACY & PHARMACOLOGY

POST-DOCTORAL RESEARCH OFFICER IN MEDICINAL CHEMISTRY

Applications are invited for the above p from graduates in Pharmacy, Chemistry Biochemistry, with postgradu qualifications and experience. T successful candidate will work w Professor R T Parfitt on Novel Centr Acting Tropanes. A good knowledge organic synthesis and modern spect scopic techniques is essential and so background in either medicinal chemis or pharmacology is desirable.

The post is tenable for two years wit starting salary of up to £5,199 per annu

Application forms and furth particulars from the Personnel Offic University of Bath, Bath, BA2 7A quoting reference number 79/217. Clos date will be 18.10.79. 3351(A

WASHINGTON UNIVERSITY SCHOOL OF MEDICINE

Assistant Professor of Microbiology

The Department of Microbiology Immunology, Washington Univers School of Medicine invites applicati from highly qualified individuals for appointment as Assistant Professor.

Applicants should have PhD or degrees, 2-5 years of post-doctoral resea experience and a commitment to med and graduate student teaching. Teach competence should be in pathoge microbiology; research interests should in microbiology.

Send curriculum vitae, brief descript of research plans and names of th referees to Dr Jospeph M. Da Department of Microbiology a Immunology, Washington Univer School of Medicine, St. Louis, MO 63 by Nov.1, 1979. Equal Opportunity Affirmative Action Employer M/F/H.

W401(A

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF MICROBIOLOGY

Applications are invited for the post of

RESEARCH ASSISTANT/ SENIOR RESEARCH ASSISTANT

in the Department of Microbiology. Candidates should hold or be about to receive, a PhD degree. The project is funded by the M.R.C. for a period of three years. The research concerns the uptake and establishment of plasmid DNA during transformation of *Escherichia coli* and other Gram negative bacteria, in collaboration with Dr G.O. Humphreys and Dr J.R. Saunders.

The initial salary will be £3,775, £4,055 or £4,333 per annum plus USS benefits. The appointment is from 1st October, 1979, or as soon as possible thereafter.

Applications, together with the names of three referees, should be received as soon as possible by The Registrar, The University, P.O. Box 147, Liverpool, L69 3BX, from whom further particulars may be obtained. Quote Ref. RV/791/N. 3304(A)

ASSISTANT PROFESSOR POSITION

DEPARTMENT OF GEOLOGICAL SCIENCES

THE UNIVERSITY OF TEXAS AT AUSTIN

A tenure-track assistant professor position is now open in the field of sedimentary geology. This position is effective January 1980 or September 1980, depending on availability of successful applicant. Teaching responsibilities include undergraduate and graduate courses in depositional processes and facies and supervision of graduate research in the area of the person's special interest.

Applicants for this position must have completed all requirements for the PhD degree at the time of employment. Qualified persons should send a complete resume including names of three references, an abstract of the dissertation, statement of research interests and planned research projects to Dr Robert E. Boyer, Chairman, Department of Geological Sciences, The University of Texas at Austin, PO Box 7909, Austin, Texas 78712. Deadline for applications: December 31, 1979. Equal Opportunity/Affirmative Action Employer, M/F. W407(A)

UNIVERSITY OF PENNSYLVANIA

THE MOORE SCHOOL OF ELECTRICAL ENGINEERING

POSTDOCTORAL POSITION

The Moore School of Electrical Engineering, University of Pennsylvania invites applicants for a postdoctoral fellowship in chemical sensing at elevated temperatures. The research will involve the investigation of semiconductor devices in high temperature operation. A background in one of the following areas is required: electrical engineering, physics or chemistry. An interest in electronics and a working knowledge of computers is desirable.

Send resume and two letters of reference to Prof. J.N. Zemel, The Moore School of Electrical Engineering, D2, Department of Electrical Engineering and Science, University of Pennsylvania, Philadelphia, 9104. An equal opportunity, affirmative ction employer. W402(A)

International Role in Quality Assurance

Health Care c.£10,000

Abbott Laboratories is among the world's leaders in the health care field, manufacturing and marketing a wide range of science-based products. We are seeking an Assistant Manager to report to the Manager of Quality Assurance covering our manufacturing operations throughout Europe, Middle East and Africa. This is a senior appointment and applications are invited from graduate scientists or engineers, male or female, with at least three years' senior management experience in quality assurance in the pharmaceutical, chemical, plastics or food industries.

The position is currently based in the U.K. but will involve extensive travel throughout the area of responsibility.

In the first instance write, giving brief details of qualifications, age, experience and current salary to:

Dr. E.M. Linday, Abbott Laboratories Limited, 42 Queens Road,
Reading, Berks. RG14 AU.

Abbott Laboratories Ltd.,

Health care worldwide

3403(A)

UNIVERSITY OF DUNDEE

DEPARTMENT OF BIOLOGICAL SCIENCES

LECTURERSHIP

Applications are invited from geneticists, microbiologists, molecular biologists and biochemists for the post of Lecturer in the Department of Biological Sciences.

Experience of nucleic acid or microbial genetic techniques would be an advantage though not essential.

Salary scale £4,333 to £8,992 (under review)

Applications (6 copies, or if posted overseas 1 copy) including a Curriculum Vitae and the names and addresses of two referees should be sent by 23 October 1979 to the Secretary, The University, Dundee, DD1 4HN from whom further details of the post can be obtained. Please quote Ref: EST/82/2/79 3353(A)

IMPERIAL COLLEGE

GRADE 4 TECHNICIAN

Required to work in fermentation pilot plant laboratory. Duties include microbiological/analytical work and operation of laboratory fermenters. The successful applicant will be required to operate a limited day shift system (0900 to 1700 hours: 6 × duty days, 3 × rest days). Salary in the range £3,432 — £3,950. (Under review 1.10.79 with further min. increase of £209 to £241 from 1.4.80). Plus London Weighting of £524 (under review). Weighting and shift inconvenience allowance.

Applications to departmental superintendent, Biochemistry Department, Imperial College, South Kensington, London SW7 2AZ. Tel: 01-589 5111 ext. 1119. 3389(A)

GUY'S HEALTH DISTRICT

BASIC GRADE BIOCHEMIST

£4,389 pa. inc. rising on an incremental scale to £5,289 pa.
(£4,818 pa. inc. rising to £5,670 pa. inc. as from January 1st 1980).

The Department of Clinical Chemistry situated in Guy's Tower has a vacancy for a biochemist. Applications are invited from suitably qualified men and women for this post, which will be for two years duration.

Staff facilities at Guy's include a staff club, swimming pool, squash courts and subsidised restaurants. Guy's is conveniently located for all public transport links at London Bridge.

For further details and an application form please contact the Personnel Department, Guy's Hospital, St. Thomas Street, London SE1 9ET or phone 01-407 7600 Ext 3462.

Closing date for receipt of completed applications: 19th Ocotober, 1979.

Lambeth Southwark and Lewisham Area Health Authority. 3366(A)

UNIVERSITY OF SURREY

DEPARTMENT OF CHEMISTRY

POSTDOCTORAL RESEARCH FELLOW

Applications are invited for a Postdoctoral Research Assistantship supported by the Science Research Council to collaborate in a research programme involving a novel approach to the selective inhibition of biosynthesis of microbial metabolites, supervised by Professor R. Thomas. Applicants should have previous experience in an area of natural product chemistry.

The post is tenable for two years from October 1979. Salary will be within Range 1A of the salary scales for Research and Analogous Staff (£4,333 to £4,910 per annum).

Applications enclosing a curriculum vitae and the names of two referees should be sent to Professor R. Thomas, Department of Chemistry, The University of Surrey, Guildford, Surrey GU2 5XH. 3385(A)

ROTHAMSTED EXPERIMENTAL STATION

Harpenden, Herts. AL5 2JQ

POPULATION GENETICIST

to work for 3 years in laboratory and field as a member of an interdisciplinary team on control strategies to delay build-up of resistance by insects to insecticides. The work, supported by the Leverhulme Trust Fund will be in the Department of Insecticides and Fungicides.

Qualifications: 1st or upper 2nd class Honours degree in Population Genetics with at least 2 years' post qualifying experience. Knowledge of computing essential. Preference will be given to postdoctoral candidates.

Appointment in grade of Higher Scientific Officer, £4,727 to £6,211; (£5,097 to £6,737 from 1.1.80). Non-contributory superannuation.

Apply in writing to the Secretary, giving names and addresses of two referees and quoting Ref. 410 by 18th October, 1979. Further particulars on request. 3372(A)

Plant Virologist

Senior Scientific Officer/
Higher Scientific Officer/
Scientific Officer

£3,289 – £7,864

Applications are invited for a pensionable post in the Plant Pathology Division of the Department of Agriculture.
The successful candidate will be expected to conduct research and provide specialist advice on plant disease with special reference to virus diseases.
The person appointed may be required to undertake teaching duties in the Faculty of Agriculture and Food Science, Queen's University, Belfast.

SENIOR SCIENTIFIC OFFICER
Over 25 and under 32 years of age on 31 December 1979 with a first or second class Honours degree in a scientific or agricultural discipline pertinent to Plant Pathology with at least 4 years' post-graduate experience in work in plant diseases.

HIGHER SCIENTIFIC OFFICER
Under 30 years of age on 31 December 1979 with an Honours degree as above and at least 2 years' post-graduate experience.

SCIENTIFIC OFFICER
Under 27 years of age on 31 December 1979 with an Honours degree as above.
Exceptionally applications may be considered from candidates over the age limit who have specialised experience.
The Civil Service Commissioners may decide to interview only those applicants who appear from the information available (including the level of academic attainment and relevant experience) to be best qualified.

SALARY SCALES

Senior Scientific Officer – £5,876 – £7,864 (£6,332 – £8,705)*
Higher Scientific Officer – £4,727 – £6,211 (£5,097 – £6,737)*
Scientific Officer – £3,289 – £5,085 (£3,591 – £5,486)*
* (salary scales from 1 January 1980)

In addition there is a non-contributory pension scheme apart from a 1½% deduction from salary to cover Widows' and Dependants' Benefits in the case of male officers.
Grading and starting salary will be related to qualifications and experience. There are prospects of promotion to Principal Scientific Officer (£7,534 – £9,646).

Please write or telephone for an application form quoting job reference SB 341/79/NN to the Civil Service Commission, Rosepark House, Upper Newtownards Road, Belfast BT4 3NR (telephone Dundonald 4585 ext 256). Completed forms must be returned to arrive not later than 18 October 1979. 3360(A)

NORTHERN IRELAND CIVIL SERVICE

SOUTHAMPTON GENERAL HOSPITAL

BASIC GRADE BIOCHEMIST

to work in connection with biochemical and nutritional problems of the elderly with the University Geriatric Medical Team based at Southampton General Hospital.

Successful applicant will be linked to Professor Barbra Clayton's Department of Chemical Pathology. Applicants should have an M.Sc in Clinical Biochemistry and also some knowledge or interest in Radio-Immune Assay techniques would be an advantage.

Salary scale £4,035 to £5,592 (increase in January).

Application forms and job description from District Personnel Department, Tremona Court, 30/32 Tremona Road, Southampton. Telephone Southampton 777222 ext. 3556/3018. Closing date 24th October, 1979. 3344(A)

UNIVERSITY OF SOUTHAMPTON

CANCER RESEARCH CAMPAIGN MEDICAL ONCOLOGY UNIT

Applications are invited for the position of post doctoral research fellow to work on mechanisms of resistance to anti-cancer drugs. Candidates should have, or be about to obtain, a PhD in Biochemistry or Pharmacology. Some experience of tissue culture would be an advantage. The appointment will be for 3 years and the successful applicant will be expected to take up his or her duties as soon as conveniently possible.

Salary: £4,333 to £5,777 p.a. on Range 1A. USS benefits.

Applications giving date of birth, curriculum vitae and the names of three referees should be sent to Mrs P. Vaughan-Smith, Staffing Section, The University, Southampton, SO9 5NH, as soon as possible quoting ref: 1099/R/Na

3328(A)

Innovative Bioscientists for Fermentation Development

The Fermentation Development Department of our Worthing site is concerned with the development of fermentation processes for the production of antibiotics and related substances. We now wish to recruit the following:

Microbiologist

The Strain Improvement Section requires an honours graduate in microbiology. A logical mind, coupled with an ability for systems innovation, is an essential prerequisite, and the successful candidate would also be expected to co-ordinate the activities of a small technical team.

Microbial Biochemist

The Biochemistry Section is involved in characterizing the critical events controlling the large scale production of antibiotics. Applicants should possess a good honours degree in biochemistry or microbiology, preferably with some post graduate experience. They should have an innovative approach combined with the flexibility of outlook necessary to deal with the varied problems arising from a complex bio-technological operation.

As well as a pleasant South Coast location Beecham offers a bracing scientific atmosphere.

Please write or telephone for an application form to:

Mr A D Caws, Senior Personnel Officer, Beecham Pharmaceuticals, Clarendon Road, Worthing, West Sussex, BN14 8QH. Telephone: (0903) 39900 Extension 233.

Beecham Pharmaceuticals 3380(A)

SALFORD AREA HEALTH AUTHORITY (TEACHING)
ROYAL MANCHESTER CHILDREN'S HOSPITAL
Hospital Road, Pendlebury, Manchester

Applications are invited from suitably qualified candidates for the post of

RESEARCH SCIENTIFIC OFFICER (BASIC GRADE)

in the Department of Chemical Pathology and Cytogenetics to work on a project involving the study of growth factors in the field of tissue culture.
The post will be for one year in the first instance, renewable for one further year, previous experience with tissue culture would be an advantage.
Gross salary scale: £3,486 – £4,527 per annum, rising on the 1st January 1980 to £3,807 – £4,824 per annum. Specific salary will be awarded according to qualifications and experience.
Informal enquiries to Doctor G. M. Addison. Telephone: 061 794 4696.
Written applications giving details of qualifications, relevant experience and the names and addresses of two referees to the Sector Administrator at the above hospital, from whom a job description can be obtained.
Closing date: 19th October, 1979.

3364(A)

ICI

ICI CORPORATE LABORATORY, RUNCORN

MOLECULAR BIOLOGIST

Applications are invited from molecular biologists with a minimum of two years' post-doctoral experience to join a research team interested in the control of expression of eukaryotic genes. Experience in the isolation of MRNA from mammalian tissue and techniques of DNA cloning are highly desirable.

The appointment will be on a permanent basis and the work will be situated in our laboratories at Runcorn where a GMAG approved category III laboratory is available. In addition, there will be opportunity to collaborate with the group working in the ICI Joint Laboratory in the Biochemistry Department at the University of Leicester.

Excellent career prospects exist and, in addition to a competitive salary, the Company has a profit sharing scheme and an excellent contributory pension scheme. Where appropriate, assistance will be given with relocation expenses.

Applicants should write, enclosing full curriculum vitae, to:—

Miss S E Bird
Personnel Officer
ICI Corporate Laboratory
P O Box 11 The Heath, RUNCORN
Cheshire

3293(A)

UNIVERSITY OF LAUSANNE

DEPARTMENT OF PHARMACOLOGY

RESEARCH ASSISTANT

pplications are invited for this post available from 1 November 1979 for two years with ossibility of renewal. Candidates (M.Sc., Ph.D, or MD) should be familiar with ochemical techniques, with experience in purification of proteins (including affinity romatography) and production of antibodies. The successful candidate will work within r hypertension research group. Knowledge of French would be an advantage but is not a quirement.

Salary within range of Sw. Frs. 27'700.--to Sw. Frs. 47'607.-- p.a.

Applicants should send a curriculum vitae and names of three referees to: Prof. G. ters, M.D., Institut de pharmacologie de l'Université de Lausanne, Rue du Bugnon 21, H-1011, *Lausanne*, (Switzerland)

W405(A)

MEDICAL RESEARCH COUNCIL NATIONAL INSTITUTE FOR MEDICAL RESEARCH

Applications are invited for the post of

HEAD OF THE ANIMAL DIVISION

in succession to Dr. J.J. Bullen who will be retiring in September 1980. The primary responsibilities of the post are the organisation and supervision of the breeding of animals and of facilities for experimental work. Facilities for research are available and can be discussed with the candidates. Preference will be given to applicants with a veterinary qualification.

The post is equivalent to that of a Professor or Reader in a university and carries the corresponding salary.

Further details are available on request.

Applications, accompanied by the names of two referees, should be sent to the Director, National Institute for Medical Research, The Ridgeway, Mill Hill, London, NW7 1AA, to arrive not later than 30 November, 1979.

3361(A)

CSIRO AUSTRALIA

Postdoctoral Research Fellow

Division of Soils
Adelaide SA

CSIRO has a broad charter for research into primary and secondary industry areas. The Organization has approximately 7,000 employees — 2,400 of who are research scientists — located in Divisions and Sections throughout Australia.

Field: Isotope Hydrology

General: The Division undertakes research on a wide range of subjects within the field of soil science aimed at conserving and managing Australia's soil and water resources for agricultural, urban and recreational use. The hydrology program is one of a number being pursued in Adelaide. It is currently concerned with the use of natural tracers for the evaluation of hydrologic parameters in a range of natural systems. With support from the Engineering and Water Supply Department of South Australia and in collaboration with Flinders University, the Division is initiating a study of the water resources of the low rainfall areas of northern South Australia. The techniques of isotope hydrology should make a unique contribution to an understanding of the hydrology of these and other arid areas.

Duties: To carry out laboratory and field studies on the variation of oxygen-18 and deuterium in soil profiles under arid conditions. Subsequently the appointee will be expected to examine other areas of arid zone hydrology.

Qualifications: A PhD or equivalent with experience in isotope hydrology together with the ability to apply mathematics to natural systems. As much if the work will be involved with the unsaturated zone, a knowledge of the principles of soil physics would also be an advantage.

Salary: Research Scientists: $A15,916 — $A19,509 pa.

Tenure: Two years.

Applications IN DUPLICATE, stating full personal and professional details, the names and addresses of at least two professional referees, and QUOTING REFERENCE NUMBER 270/490 should reach:—

The Personnel Officer, Australian Scientific Liaison Office, Canberra House, 10-16 Maltravers Street, London WC2R 3EH, by 6th November 1979

Applications in U.S.A. and Canada should be sent to:— The Counsellor Scientific, Embassy of Australia, 1601 Massachusetts Avenue, N.W., Washington, D.C., 20036, U.S.A.

3414(A)

BIRMINGHAM MATERNITY HOSPITAL

Central Birmingham Health District

BASIC GRADE CYTOGENETICIST

Applications are invited for this post from persons with a relevant science degree. Experience in human cytogenetics is desirable.

The successful applicant will work in the Cytogenetics Laboratory in the Infant Development Unit at Birmingham Maternity Hospital.

Further details and permission to visit the laboratory may be obtained from Mrs S.M. Bowser-Riley (021 472 1377 Ext. 157) Principal Cytogeneticist, Infant Development Unit, Birmingham Maternity Hospital, Birmingham B15 2TG.

Application forms available from Personnel Officer at the Maternity Hospital.

3392(A)

BIOLOGICAL SAFETY OFFICER

UNIVERSITY OF FLORIDA

This position requires a Master's Degree in an area of Biological or Basic Medical Sciences with a minimum of 3 years experience in biological safety. Experience should include basic scientific techniques and manipulations common to biomedical research. A technical knowledge in basic microbiology and biological safety techniques and practices, containment equipment, and engineering principles pertaining to the design and operation of biological and medical facility safeguards is also required.

Send complete resume with salary requirements and salary history by November 15, 1979 to:

Robert L. Willits
Administrative Employment Manager
Second Floor HUB
University of Florida
Gainesville, Florida 32611

EQUAL EMPLOYMENT OPPORTUNITY/
AFFIRMATIVE ACTION EMPLOYER

W414(A)

FELLOWSHIPS/ STUDENTSHIPS

UNIVERSITY OF KEELE

DEPARTMENT OF PHYSICS

RESEARCH FELLOWSHIP

Synchrotron Radiation Protein Crystallography

Applications are invited for a three year SRC supported Post Doctoral Fellowship commencing on 1st October or as soon as possible thereafter to work with Dr J.R. Helliwell on the x-ray crystallographic study of 6-phosphogluconate dehydrogenase from Bacillus stearothermophilus utilising the Synchrotron Radiation Source (SRS) presently under construction at Daresbury Laboratory as well as facilities within the Department. Initial experiments, prior to the opening of the SRS, will be performed at other synchrotron radiation facilities in Europe.

The successful applicant will also be involved in the setting up of the protein crystallography experimental apparatus at Daresbury. Experience in crystallography, X-ray scattering or synchrotron radiation advantageous but not essential.

Salary starts at point 3 of the research scale 1A £4,910 to £5,488. Further particulars and application forms from The Registrar, The University, Keele, Staffs., ST5 5BG to whom completed forms, as well as curriculum vitae and details of research experience, should be returned as soon as possible. 3383(E)

McMASTER UNIVERSITY

DEPARTMENT OF BIOCHEMISTRY

A POSTDOCTORAL FELLOWSHIP

is available in the area of mitochondrial biogenesis in mammalian cells with emphasis on genetics, proteins biosynthesis and membranes, with Dr K.B. Freeman.

The fellowship is valued at $12,200 per year, taxable and may be renewed for one year. For further information write to: Dr K.B. Freeman, Department of Biochemistry, McMaster University, 1200 Main Street West, Hamilton, Ontario L8S 4J9 Canada. W396(E)

-Unique Opportunity- STANFORD UNIVERSITY

NSF POSTDOCTORAL FELLOWSHIP in MOLECULAR BIOLOGY and COMPUTER SCIENCE

$14,000 per annum

Applicants should be familiar with the techniques of modern molecular biology and be interested in entering the field of computer science. Previous programming experience is useful but not essential. Responsibilities will include development and maintenance of a knowledge base of current laboratory techniques for use with an advanced model of automated experimental planning. One goal of this program is to train an individual for a career in computer science as applied to molecular biology and genetics. While the fellowship will be awarded through the Department of Computer Science, the fellow will interact regularly with research groups in the Departments of Biochemistry and Medicine.

Send resume and the names of three references to either:

Dr Douglas Brutlag, Department of Biochemistry or Dr L.H. Kedes, Department of Medicine, Stanford University, School of Medicine, Stanford, California 94305. Equal opportunity through affirmative action. W383(E)

Southampton THE UNIVERSITY

DEPARTMENT OF CHEMISTRY

Applications are invited for a CASE Studentship, co-sponsored by Bush, Boake, Allen Ltd. The project is concerned with Organometallic Complexes and Catalysed Reactions of Isoprene, and the successful candidate will be expected to register for a higher degree. Applicants should have at least a University Upper Second class degree.

Applications, including the names of two referees should be sent to: Professor R. Baker, Department of Chemistry, The University, Southampton SO9 5NH. 3384(F)

THE UNIVERSITY OF SYDNEY

F. H. LOXTON POSTGRADUATE STUDENTSHIPS

Applications are invited for the above studentships from male graduates who wish to undertake postgraduate research in the following areas:

AGRICULTURE CHEMICAL ENGINEERING

Areas of current interest in the Department of Chemical Engineering include: Biochemical Engineering, Computer-Aided Design, Computer Process Control, Distillation and Mass Transfer, Environmental Engineering, Minerals Processing, Particulate Solids Technology, Petroleum and Reservoir Engineering, Reaction Engineering, Renewable Energy Sources, Solar Energy, including fuel alcohol production and desalination technology, Solids and Particle Technology and Systems Engineering.

VETERINARY SCIENCE

General

The studentships are tenable for one year and may be renewed for up to three further years and are valued at $A4,200 per annum plus allowances. Further information is available from: The Registrar, the University of Sydney, NSW 2006, Australia, with whom applications close on 20 December, 1979. 3390(F)

The ISAAK WALTON KILLAM POSTDOCTORAL FELLOWSHIPS

1980-81

DALEHOUSIE UNIVERSITY

Value

Fellowships valued at $13,500 (1979-80), plus travel allowances, are tenable for one year at Dalhousie University in most fields of study. Application for extensions may be considered.

Qualification

applicants should have recently completed a PhD Degree at a recognized university, or expect to obtain a degree before taking up an award.

Application

Application forms may be obtained by writing to:

The Office of the Dean, Faculty of Graduate Studies, Dalhousie University, Halifax, Nova Scotia B3H 4H6

The closing date for receipt of applications is 15 December, 1979. W382(E)

ATHROFA GOGLEDD-DD CYMRU

THE NORTH E WALES INSTITUTE OF HIGHER EDUCATION

Kelsterton College, Connah's Quay, Deeside, Clwyd

SRC CASE STUDENTSHIPS

Applications are invited for a SRC CASE Studentship tenable immediately for work on the following project: "Radio-immunoassay of polyamines", in collaboration with Ortho Diagnostics, Ltd. Supervisors: Drs. J. C. Allen and C. J. Smith.

The co-operating body will supplement the SRC Studentship by £250 p.a. Applicants should possess or expect to obtain 1st or upper 2nd class Honours, and should write as soon as possible, with curriculum vitae, to the Institute Registrar, North E Wales Institute, Kelsterton College, Connah's Quay, Deeside, Clwyd CH5 4BR. 3395(F)

UNIVERSITY OF COLORADO

VISITING FELLOWSHIPS

For Research in Solid Earth and Atmospheric Science

One year awards offered by CIRES, a Research Institute sponsored by NOAA and the University of Colorado. Stipend is scaled to experience, and the program is open to scientists of all countries.

Applications for the 1980-81 academic year awards should be sent to The Director, CIRES Visiting Fellows Program, Campus Box 449, University of Colorado, Boulder, Colorado 80309 USA, before February 1, and should include names of three referees, a vitae and publications list, and a brief outline of the proposed research.

Affirmative Action Equal Opportunity, Section 540, Employer. W399(E)

ASSISTANTSHIPS

THE UNIVERSITY OF BRITISH COLUMBIA

DEPARTMENT OF CHEMISTRY

GRADUATE TEACHING ASSISTANTSHIPS 1980/81

Academic Year

Graduate Teaching Assistantships are available for candidates with high academic records who wish to pursue further studies towards the M.Sc. and PhD Degrees in Chemistry. Excellent research facilities are available in all areas of modern Chemical Science, ranging from Bio-Inorganic Chemistry to Chemical Physics. For first year students the minimum stipend, including the summer research assistantship, is anticipated to be $8,000 per annum.

Students entering the programme with a Master's degree will receive a higher minimum stipend of $8,500 per annum. In addition, many special merit awards are made to those graduate teaching assistants who show excellence in the performance of their duties. Numerous graduate scholarships are also available for students with outstanding academic records.

Application forms and further particulars may be obtained from the undersigned:

Professor C.A. McDowell,
Department of Chemistry, The
University of British Columbia, 2075
Wesbrook Mall, Vancouver, B.C.
Canada, V6T 1W5 W400(P)

UNIVERSITY OF DURHAM

DEPARTMENT OF PHYSICS

Applications are invited from both

THEORETICIANS and OBSERVERS

for an SRC funded

POSTDOCTORAL SENIOR RESEARCH ASSISTANTSHIP

tenable for 2 years from 1 January 1980 to work in Extragalactic Astronomy with the Cosmology group.

Initial salary in the range £4,333 to £4,910 on Range 1A plus superannuation.

Application (3 copies) naming three referees should be sent by 19 November 1979 to the Registrar and Secretary, Science Laboratories, South Road, Durham, DH1 3LE, from whom further particulars may be obtained. 3357(P)

UNIVERSITY OF DURHAM

DEPARTMENT OF PHYSICS

Applications are invited from a physicist or applied mathematician, with experience in advanced numerical methods, or in the use of large and mini computers for a

SENIOR RESEARCH ASSISTANTSHIP

tenable for three years from as soon as possible. He or she will be a member of the High Energy Cosmic Ray Group and will be responsible for the development of analysis methods for data from atmospheric Cerenkov radiation experiments in the U.S.A. Furthe information may be obtained from Dr K.E. Turver or Dr K.J. Orford (Te Durham 64971).

Salary in the range £4,333 to £7,521 pe annum plus superannuation.

Applications (3 copies) naming thre referees should be sent by 31 October 197 to the Registrar and Secretary, Scienc Laboratories, South Road, Durham DH 3LE. 3365(P)

GRANTS and SCHOLARSHIPS

THE ROYAL SOCIETY

THE RUTHERFORD SCHOLARSHIP

This award, for graduates of exceptiona promise and ability who are under 26 yea of age on 1 May 1980, is for experimenta research in any branch of the natura sciences, with a preference fo experimental physics if there are candidate of similar merit. It will be tenable for thre years, from 1 October 1980 or such othe date as may be arranged, in some part of t British Commonwealth other than that which the applicant graduated. The valu adjusted to meet the circumstances, will b £2,250 per annum on appointment (und review) if held in the United Kingdom, wi allowances for travel, university fees, etc. will not normally be awarded to a gradua who has already held a senior researc award.

Applictions should be made as follows

From university graduates *in the Unit Kingdom*: before 15 February 1980 forms obtainable from the Executi Secretary, The Royal Society, 6 Carlt House Terrace, London, SW1Y 5AG.

From university graduates *outside t United Kingdom*: through their universiti to the Royal Commission for t Exhibition of 1851 on forms of applicati for the Commissioners' Science Resear Scholarship to be obtained from t registrars or administrative authorities their universities. 3371(H)

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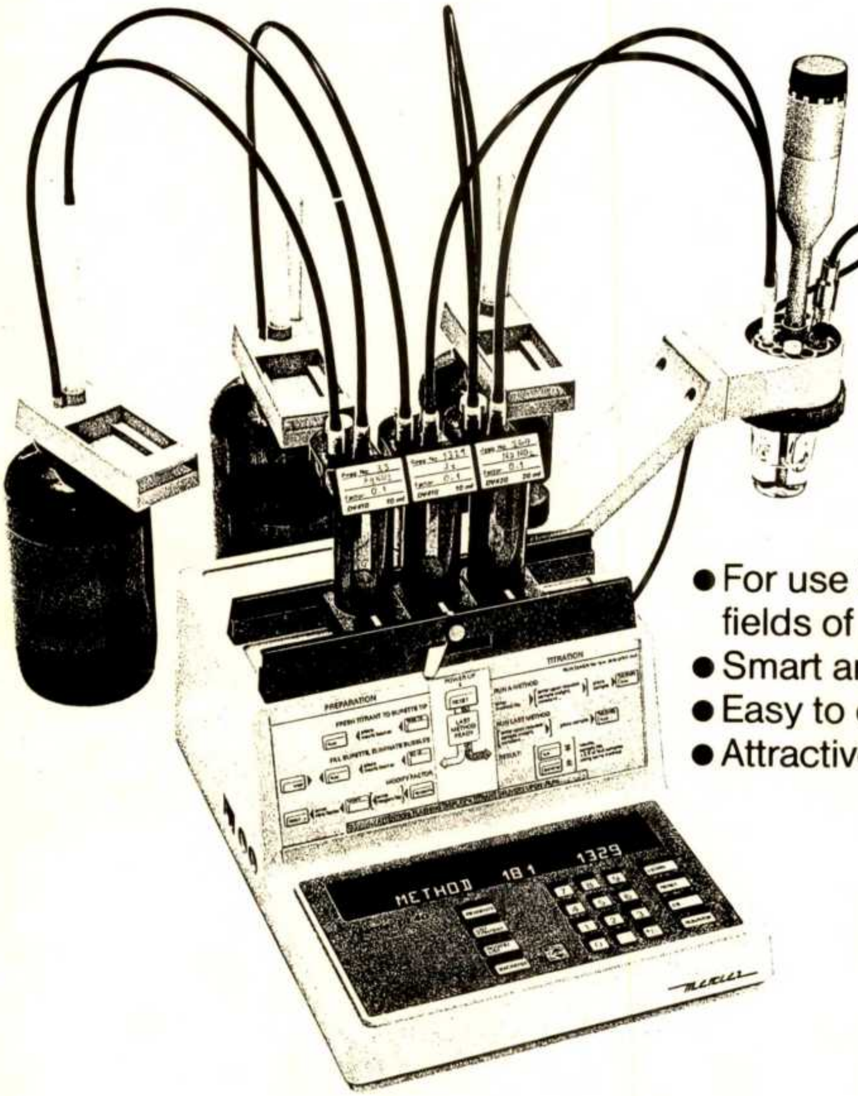

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The Second Translation Kit

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The First Translation Kit

It will make your life easier and your results more reproducible

New England Nuclear offers the first reticulocyte lysate kit for use in studying protein synthesis. Not only does it save all of the trouble of preparing your own cell extract, it gives you a system free from endogenous mRNA, high in protein synthesis activity, with an optimized high-activity tracer and quality control that includes testing every lot of Methionine, L-[35S] using the kit.

The rabbit reticulocyte lysate system is reported to contain all factors required for translation of any eukaryotic mRNA! But its use assumes the availability of suitable animals, preparation of the lysate extract, and inactivation of endogenous mRNA. And the meticulous tailoring of the salts, energizer, and amino acid mix. In short, it's a nuisance.

With our exclusive kit you bypass all that and concentrate on the study of protein synthesis. The kit contains:

A rabbit reticulocyte lysate—Treated with nuclease to inactivate endogenous mRNA, and tested to guarantee high protein synthesis activity.

The first tracer available—NEG-009T, NEN's translation grade [35S]-Methionine, >500Ci/mmol, tested for incorporation into protein in cell-free translation systems. Each lot is optimized for compatibility with the system.

Translation cocktail—All the necessary components for proper translation conditions: 19 amino acids (excepting methionine) plus buffer, reducing agent, and energizer.

Control mRNA—Purified exogenous mRNA (adenovirus-infected HeLa cell mRNA) to check the biological activity of the system.

Salts and sterile deionized water—Selected and prepared to minimize potential ionic or enzymatic inhibition of the system.

And you can get rid of all those rabbits

The protocol is straightforward, and it includes suggested final assay procedures. So that's our message. Now add yours.

Call us toll free for details.

New England Nuclear

First, Methionine...

Our first translation kit saved you the difficulty of preparing your own lysate for cell-free translation studies, gave you a system free of endogenous mRNA and high in protein synthesis activity, and assured you of components tested for compatibility and reproducible results. All told, it was an excellent excuse to stop cultivating rabbits.

Our second kit does all of the above, too. But, instead of Methionine, L-[^{35}S], the tracer is Leucine, L-[3,4,5-^{3}H]—specially optimized for use with this system. Here's what our Leucine Translation Kit contains:

Rabbit reticulocyte lysate Treated with micrococcal nuclease to inactivate endogenous mRNA and tested to guarantee high protein synthesis activity.

High activity ^{3}H tracer NET-460T, Leucine, L-[3,4,5-^{3}H], >110Ci/mmol. Special lots are prepared and tested for incorporation into protein using this cell-free translation system.

Translation cocktail All the necessary components for proper translation conditions, including buffer, biogenic polyamine, and energizer.

Control mRNA Purified mRNA to check the biological activity of the system.

Salts and sterile deionized water Selected and prepared to minimize potential ionic or enzymatic inhibition of the system.

Caution: Not for use in humans or clinical diagnosis.

Instructions for use The protocol is straightforward and includes suggested assay procedures.

So now there are two, and only two, translation kits. Call us toll-free for details on either or both.

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Daimlerstrasse 23, Postfach 401240, Telephone (06103) 85034, Telex 4-17993 NEN D
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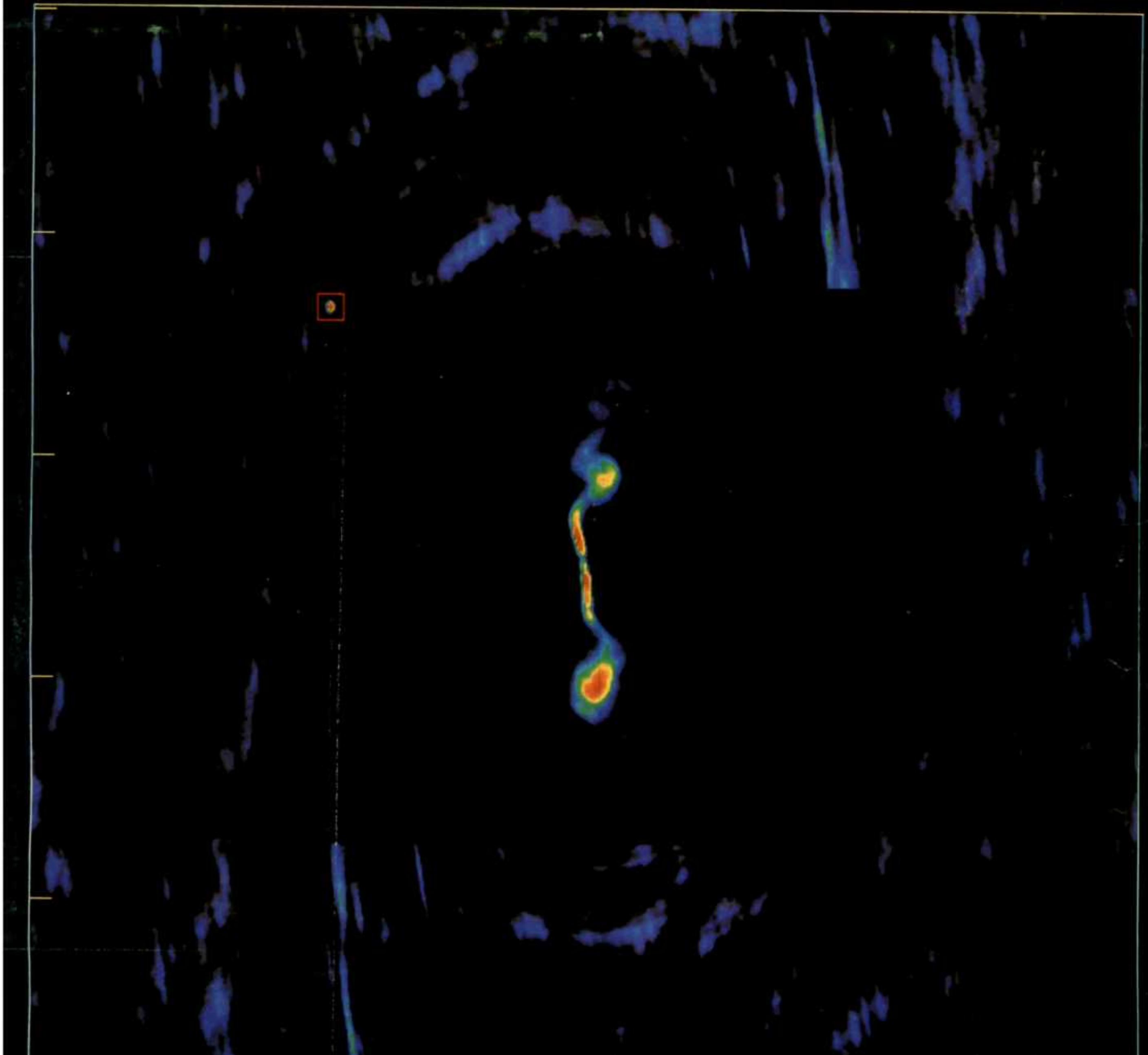

Galactic Radio Jets

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Whenever the cost/price squeeze gets really tough, it's a temptation to regard advertising as a cost...and to cut.

Not at every company, however.

In recent years, a significant change has taken place in the thinking of many management men about advertising budgets. No longer are appropriations cut automatically when the pressure is on.

Why?

For a number of reasons. Among them are:

1. *With the growth of the marketing concept,* advertising is no longer looked upon merely as an expense, but as an integral part of the company's marketing mix.

2. *Firms that maintain advertising during recession years do better in sales—and profits—in those and later years.* That was proved conclusively in studies of five separate recessions made by ABP and Meldrum and Fewsmith.

3. *The cost of a salesman's call today makes it imperative to make maximum use of advertising.* The average cost of an industrial sales call soared to a record $96.79 according to the latest report by McGraw-Hill's Research Laboratory of Advertising Performance. Yet studies show that a *completed* advertising sales call—that is, one ad read thoroughly by one buying influence—literally costs only pennies. Why deny yourself such efficiency?

4. *In some cases, there is no way to reach customers except by advertising.* The "Paper Mill Study" shows (1) the number of buying influences in the average plant is far greater than marketers are aware of, (2) the vast majority of these influences are unknown to salesmen, (3) no salesman has the time to contact all influences even if he knows them.

5. *Selling costs are lower in companies that assign advertising a larger role in marketing products.* So advertising is an investment in profit, just like a machine that cuts production costs.

6. *Memories are short.* There is an estimated 30% turnover every year among buyers. It isn't surprising, then, that lack of advertising contact can quickly result in loss of share of market.

7. *Most down periods turn out to be shorter than expected.* The history of every postwar recession is that it didn't last as long as predicted. Why gamble your market position for short-term gain?

8. *Consider lead time.* Very few products sold to business and industry are bought on impulse. The advertising you are doing—or missing—right now will have its effect years from now.

9. *Advertising works cumulatively.* It would be nice to think that every reader reads all of your ad. We know it doesn't work that way. To be most effective, advertising must have continuity.

10. *Did your competitor cancel his budget, too?* If not, you may be taking a big risk.

11. *Will you lose salesmen?* They know that their chance of getting an order is better if they are backed up by advertising. Can you be sure of keeping them when they learn that that support has gone?

12. *You know better.* Survey after survey of executives shows that they expect a *drop* in sales if advertising stops.

But there is need for efficiency...

whenever advertising budgets are being assembled—never more than in these inflationary times. Significantly, a recent survey shows that nearly 40% of the average budget for advertising to business and industry is invested in business publication space and preparation. That's *more than double* the next largest item.

Why? Because specialized business publications remain the most effective and efficient method of reaching target audiences in business, industry and the professions.

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Editor: David Davies
Deputy Editor: Peter Newmark

Editorial Staff
Philip Campbell Sara Nash
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Mary Lindley Charles Wenz
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Publishing Director: Christopher Paterson

New York
175 Fifth Avenue, New York, NY 10010
Telephone: (212) 777 5858 Telex: 668497
Robert Ubell (American Publisher)
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Washington News Bureau
711 National Press Building, DC 20045
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Cover picture

False-colour radio map of the galaxy 3C449. A detailed study of the radio jets, obtained with the Very Large Array in Mexico, is described on pages 437-442.

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Abbreviations should follow the *World List of Scientific Periodicals*, fourth edn (Butterworth, 1963-65). Symposia are often difficult to refer to and only published or soon-to-be-published volumes should be mentioned in references. Their publisher and place of publication should be clearly indicated. 'Personal communication' and 'unpublished' should be incorporated in text.

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applied excellence

Advanced techniques in routine use at The Radiochemical Centre

Described below are just two examples of the many up-to-date techniques, which have been pioneered or applied for routine use at The Radiochemical Centre. These developments are part of our constant endeavour to maintain our position at the forefront of the specialised field of tracer methodology, so that we can continue our supply of radiochemicals of the highest quality and technical specifications.

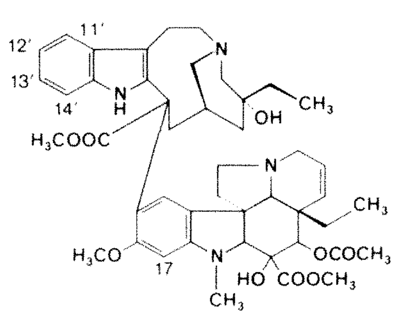
Distribution of labelling in tritium compounds

Modern techniques for the production of tritiated compounds are more sophisticated than those used in the early days of tritium labelling, and produce compounds labelled in specific positions rather than generally labelled. Nevertheless, it is necessary for many tracer applications of tritium compounds to know the *precise* position and configuration of the tritium labels. Traditional chemical methods of doing this are tedious and time consuming and subject to considerable error, and so the routine supply of such information has until recently not been possible.

The Radiochemical Centre, in collaboration with the University of Surrey, has developed over the past eight years the technique of tritium nuclear magnetic resonance (tnmr) spectroscopy for this purpose. This method is much quicker and more accurate than the traditional chemical or biochemical methods for determining distribution of tritium labelling.

It is now used routinely to establish the distribution of tritium labelling produced by the usual methods of tritiation employed at The Radiochemical Centre. We supply accurate details as to the position and configuration of the tritium labels for an increasing number of our labelled compounds.

A number of examples are given below:



[G-^{3}H]VINBLASTINE
Code TRK.507
17 .... 44% 11' 8%
12',13' .... 46% 14' 2%

[1ß, 2ß(n)-^{3}H]TESTOSTERONE
Code TRK.162
1∝ .... 5.9% 2∝ 8.2%
1ß .... 46.0% 2ß 39.9%

[6-^{3}H]BENZO[a]PYRENE
Code TRK.501
6 .... >95% 1 .... <5%

D-[2-^{3}H]GLUCOSE
Code TRK.361
2(∝) .... 40% 2(ß) 60%

L-[3,4(n)-^{3}H]VALINE
Code TRA/K.533
2 .... 8% 4 .... 64%
3 .... 28%

The latest of our publications is as follows:
AL-RAWI, J.M.A., BLOXSIDE, J.P., ELVIDGE, J.A., JONES, J.R., CHAMBERS, V.E.M., CHAMBERS, V.M.A. and EVANS, E.A., *Steroids* vol. 28 (3), p.p. 359-375, 1976.

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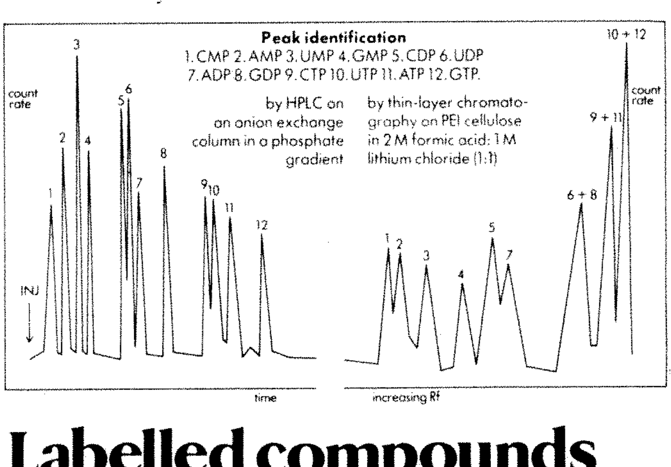

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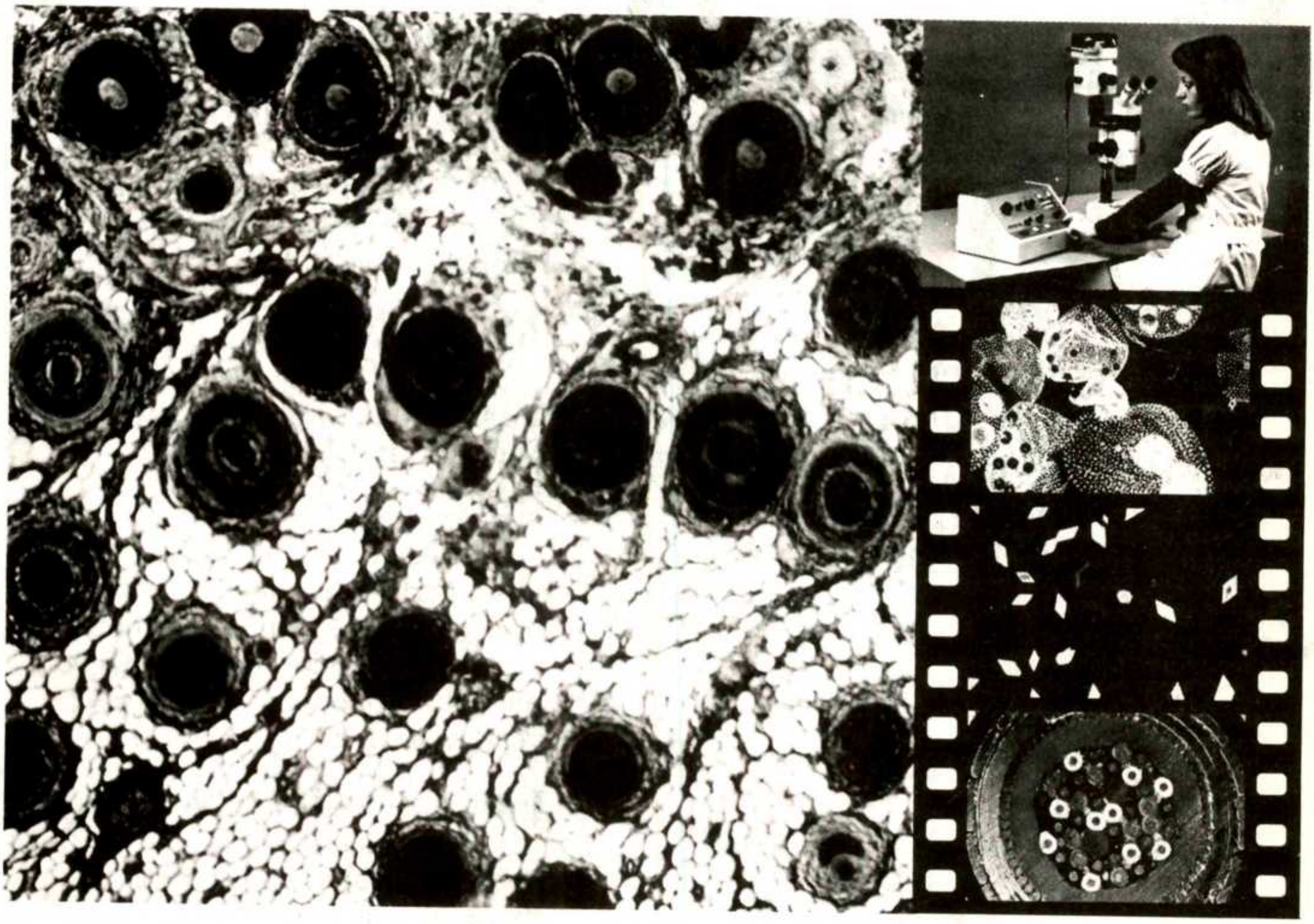

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nature
11 October 1979

Cambodia's long path back

Relief operations in Cambodia must be based on accurate information, of which there is little. And a new scientific and medical community will have to be developed.

AT LONG last there seems to be some movement on the diplomatic front over the tortured question of aid to Cambodia. Announcements of a British government provision of more than £4 million in medical and food aid, together with news that Oxfam is starting to take supplies into the country, do at least represent a step forward from the days of wretched international diplomacy in which the precise status of Cambodia's two claimants to power seemed a matter of more concern to the Western countries most capable of helping than did the fact that millions were starving to death. But now in the euphoria that at least something is happening in the short-term, attention must not be diverted from the long-term needs of the area.

Cambodia, or Democratic Kampuchea as it has recently been called, is a very fertile area. The story is told by Prince Sihanouk that US AID officials once visited Cambodian villages to encourage the planting of higher yield varieties and came back the next year not to find bumper harvests but that the villagers were now people of leisure, being able to fulfil all their needs on only half the land. All the more galling then that the successive disruptions of the past ten years — the US bombing, three years of the Pol Pot regime and then the Vietnamese invasion during the planting season in late 1978 — should have brought the country to its present position.

The problem with dispatching aid in the past few months has been the legitimacy issue. Vietnam-supported Heng Samrin has driven the Pol Pot forces into the more inaccessible parts of the country, and by the general rules of diplomacy (as applied for instance to Iran and Uganda) would probably qualify for recognition. But Pol Pot is supported by China, whereas Heng Samrin ultimately receives support from Moscow. And at present China is being courted by so many countries that when the question of recognition of a Cambodian government came up recently, the United Nations voted strongly in favour of seating the Pol Pot regime.

Thus, when the country desperately calls for international assistance there is general confusion. Cool logic suggests that the aid should go by way of Phnom Penh, the capital in the hands of Heng Samrin. But the involves at least some sort of acknowledgement of Heng Samrin's legitimacy — particularly difficult for Britain to swallow since Mrs Thatcher made it clear that she would do nothing to help Vietnam. There are, furthermore, always those who can derail a relief operation by claiming that the assistance will simply fall into the hands of the army, which some of it undoubtedly will.

Mercifully the British government has at last made a substantial step forward, and other countries seem in the process of doing so also. The question then remains of how this avalanche of aid will be put to good use. Observers of the country stress how little is really known of Cambodia's plight. Even the population is a matter of conjecture, estimates varying between 3 and 6 million. Such vital information as the prevalent type of starvation, and the distribution of people — are they in relief camps around the cities, or in scattered villages? — is barely known. And yet the character of relief operations depends crucially on this knowledge. One wonders, parenthetically, whether the United States and Soviet Union, always prepared to launch a satellite to keep an eye on other people's troubles, have an adequate supply of pictures to answer many of the questions concerning population distribution, how they are proposing to make this information available, and if not, why not.

If there are short term problems of the intelligent deployment of aid, there are, equally, long-term problems for the country. To regain its status as the rice bowl of Asia, a vigorous agricultural policy will have to be evolved in the next few months. The last planting, in war-time conditions, only used 10% of the available land — getting back to more normal land usage will not just happen automatically. Nor will medical services revive without much assistance. Estimate of the number of doctors who have survived range between 40 and 50 — a tenth of the figure of a few years ago.

If Cambodia's agricultural and medical community is to be rebuilt properly, a major international operation must be set up very soon to speed the training of qualified manpower. This will need concerted effort by many nations; it is to be hoped that the diplomatic and political considerations which have bogged down relief plans will not prevent institutions in the West from helping in the recovery of a shattered country.

● While governments are beginning to act, there is still enormous scope for charities with their greater flexibility. A UK appeal through the normal channel of the Disasters Emergency Committee has not met with a very generous response. Two charities which do seem to be capable of responding to local needs are Oxfam, Banbury Road, Oxford, and Medical Aid for Cambodia, 36 Wellington Street, London WC2. □

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Military in clash over US nuclear fusion research

US physicists claim that the use of heavy ion beams is now a leading contender for commercial fusion technology — but that research efforts are being hampered by the aspirations of the military establishment. **David Dickson** reports

TENSIONS are growing between the Carter administration and the US Congress over the appropriate strategy for pursuing one of the most promising paths towards a commercially-viable nuclear fusion energy programme — namely the use of inertial confinement techniques, which would harness the energy released by fusion during the implosion of a small hydrogen pellet hit by a converging beam of immense power.

The prime cause of this tension is a dispute over whether, since such techniques can be applied to the detonation of nuclear weapons, all research on inertial confinement should be conducted as a military project, or whether the potential civilian applications would be better served through a separate non-classified programme.

Behind this is a more conventional dispute involving competition over access to and control over limited research funds between different national laboratories. Here the current control of inertial confinement research by the two weapons laboratories run by the University of California under contract from the Department of Energy — the Lawrence Livermore Laboratory and the Los Alamos Scientific Laboratory — is being challenged by physicists from some of the major accelerator laboratories. In particular, these include the Argonne National Laboratory in Illinois, the Brookhaven National Laboratory on Long Island, and the Lawrence Berkeley Laboratory in California.

The physicists are particularly concerned that if, as Congress is now demanding, one of the weapons laboratories is designated the lead laboratory for inertial confinement research, this could penalise their own efforts — even though, they claim, these may have the best chance of achieving commercial viability.

Research has been carried out since the early 1960s at the two weapons laboratories on the possible use of laser beams to ignite the hydrogen pellets — a path to fusion which is beginning to compete with more conventional programmes, such as the magnetic confinement techniques being studied at Princeton University.

Work at Lawrence Livermore has concentrated on the use of glass lasers, and has been carried out on a variety of machines of increasing power, the construction of the latest, NOVA, having begun this summer. At Los Alamos, research has been conducted in parallel on the possible use of carbon dioxide gas lasers, and the laboratory is also constructing a large experimental machine known as ANTARES.

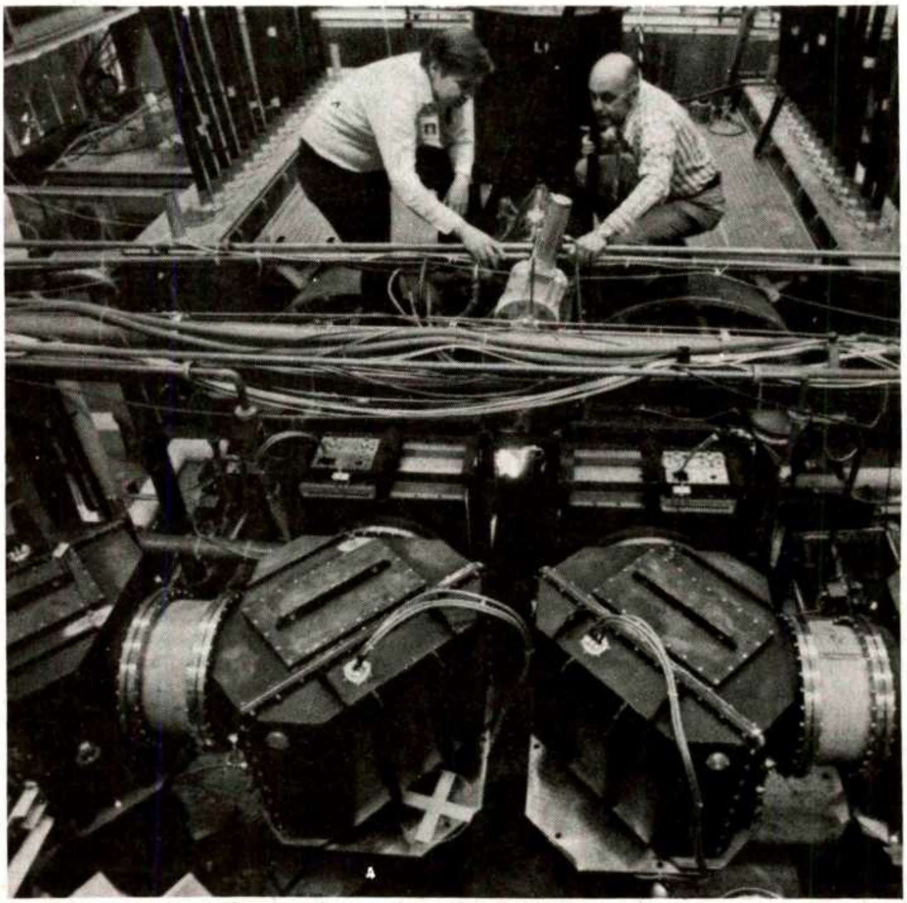
Within the past few years, however, high energy physicists have begun to explore a different approach, using particle beams to provide the necessary 'driving' impact on the pellet. In particular they have considered the use of heavy ions created by the particle accelerators already in use for basic physics research. (See *Nature* **276**, 19-23; 1978).

These physicists claim that two reports — both as yet unpublished — support their contention that there is growing disenchantment with the use of lasers for commercial fusion technology, and that particle beams in general, and heavy ion beams in particular, may offer the most practical solution. But they also claim that since heavy ions have no direct military applictions — there is no way to put an accelerator within a nuclear weapon — their research is not receiving the support it merits.

Strong support for the commercial prospects for heavy ion beam research is said to come from a report prepared for the Department of Energy by Dr John Foster, Vice President for Research at TRW Inc. Although the full report is yet to be published by the DoE, it is said to back-up Dr Foster's statement to the department's Energy Research Advisory Board in May that he considered the use of heavy ion beams to be the "first candidate" for a conservative approach to a workable fusion technology.

The second report has been prepared for the Electric Power Research Institute by a committee chaired by Dr Keith Brueckner, of the University of California, San Diego. Dr Brueckner's report does not make any comparison between the various driver technologies now under study; but it is said to confirm the Foster committee's view that there appear to be no insurmountable obstacles to the use of heavy ion technology. "The current level of funding for heavy ion beam research certainly seems to be incompatible with its promise as a commercial technology," Dr Brueckner said last week.

Both of these reports are now being considered by the Department of Energy as it prepares its budget submissions for fiscal year 1981, which President Carter will submit to Congress next January. And Dr Frank Press, director of the Office of Science and Technology Policy, is said to be among those who have been pushing the department to develop a separate civilian inertial confinement research programme,



Laser research at Los Alamos — but are they off beam?

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presumably with a greater emphasis on particle beam research.

Such moves, however, are currently being resisted by Congress, and in particular by the House Armed Services Committee, members of which argue that most of the physics of pellet design and energy transmission yet to be understood is common to both military and civilian needs. There would therefore be no need yet to split the two aspects of the research — indeed this could lead to unnecessary duplications of effort.

In passing the DoE's budget request for 1980, the committee therefore directed that the whole of the inertial confinement programme should remain conducted through the weapons laboratories — and added the rider that no money should be provided for specific studies of the commercial applications of inertial confinement fusion, or for "activities which will not be carried out in direct support of the ICF programme leading to military applications."

The committee's feelings were reflected in its reaction to the DoE's budget request. Initially, the department had requested that for 1980, about $3.8 million out of a total budget for inertial confinement research of $146 million should be allocated to heavy ion beam research at the accelerator laboratories.

Following the recommendations of the Foster committee, however, which both pointed out some of the technical limitations facing the expansion of the laser programmes and recommended a major increase in funding for particle beam research, the department suggested some rearrangements in the fusion research budget which would have increased the amount spent on heavy ion beam research to between $12 and $15 million. The suggestion was rejected by the House Armed Services Committee, however, which cut the request back to little higher than its original level.

Keeping options open

Scientists at the two weapons laboratories are still confident that they are justified in concentrating their attention on the physics of glass and gas lasers — even from the point of view of eventual civilian energy production. "It's much too early to make any assessment about the relative merits of the different systems. These are experimental programmes, and progress will only be made through experiments," says Dr Charles Fenstermarcher, programme manager for the advanced driver development at Los Alamos.

"There are many problems in the physics that are not yet understood. And you do not want to drop any option until it is certain that it is not an option."

Most physicists agree with both the DoE and with Congress that it is likely to be several years before a clear idea emerges of the most practical direction for inertial confinement as an energy technology. The problem is that, for each of the possible alternatives, generating the data required to make such an assessment involves considerable capital outlay — and therefore results in competition for relatively scarce funds.

Already Livermore's NOVA, which had originally been scheduled for completion in 1983, and for which Congress had authorised the full construction costs of $195 million, has been cut back following the recommendations of the Foster committee, and is now proceeding in two stages, construction of the first of which started in May.

Physicists at the accelerator laboratories hope to catch up — and possibly to pass — the weapons laboratory researchers in two stages, first by carrying out a joint accelerator technology demonstration programme to show the feasibility of their ideas, and subsequently, if this is successful, to move to a larger-scale machine capable of carrying out target experiments.

The first step in this process would include a substantial commitment to heavy ion beam research in the 1981 budget, possibly in the region of $20 million, with the bulk of the funds going to Argonne and Berkeley, and the remainder distributed between the other laboratories.

The next stage would obviously be more expensive. Designs have already been produced for a heavy-ion demonstration experimental facility (HIDE), which would be capable of reaching energies of 100 kilojoules, but could cost more than $100 million (and theoretically it might make more sense to go directly to an even bigger machine, costing about $500 million).

Shortage of funds could, furthermore, be accompanied by a shortage in qualified research scientists. The House Armed Services Committee points out in its report that "since this is a relatively new technology, the supply of such people is small"; accelerator scientists argue this may be one reason the weapons laboratories are reluctant to see a proliferation of inertial confinement research programmes outside their control.

They also claim that the fact that much of the current research must remain classified is one hindrance resulting from a military-controlled programme — arguing that it should still be possible to deal with the classification of potentially sensitive details even within a programme directed outwards non-military goals (as happened, for example, in the early stages of the nuclear power programme).

But whether the administration will be able to persuade Congress of its claim for the need for a separate civilian inertial confinements programme, in the face of a military establishment which sees such a programme as undercutting its own interests in such research, remains an issue that will be fought out bitterly in the months ahead. □

Congress boosts spending on health research

THE US Congress has agreed to increase the research budget of the National Institutes of Health by 8.2% to a total of $3.4 billion for fiscal year 1980. This is despite President Carter's contention in his budget request at the beginning of the year that since the NIH had received a budget increase of almost 25% in the current year, no further increase was necessary.

In discussing their separate versions of the Department of Health, Education and Welfare's Appropriations Bill, representatives in both the Senate and the House agreed to a total NIH budget which includes recommended increases that had previously been made by both sides. In particular, this will mean that for the first time the budget of the National Cancer Institute will exceed one billion dollars next year. It will also mean an increase of $26 million, as suggested by the House, for the National Institute of Arthritis, Metabolism and Digestive Diseases.

Representative Boland: dispute over Galileo

In a separate action, Senate and House conferees agreed on a budget for the National Science Foundation of $996 million dollars for 1981. Although less than the President's request for $1,006 million, this still represents an increase of 9.3% over the current year's budget.

The conferees also agreed on a budget increase of 8.2% for the National Aeronautics and Space Administration, including a 9.9% increase as requested by the Administration for research and development. Final action on NASA's budget, however, has been held up by a row between the two legislative bodies over future funding for Project Galileo, a proposed two-spacecraft mission to Jupiter.

The House, at the recommendation of representative Edward Boland, wants its appropriations committee to have the power to veto any proposals to split the mission — which may be necessary as a result of delays to the Space Shuttle. The Senate has refused to accept the condition and NASA's 1980 budget has been held up. □

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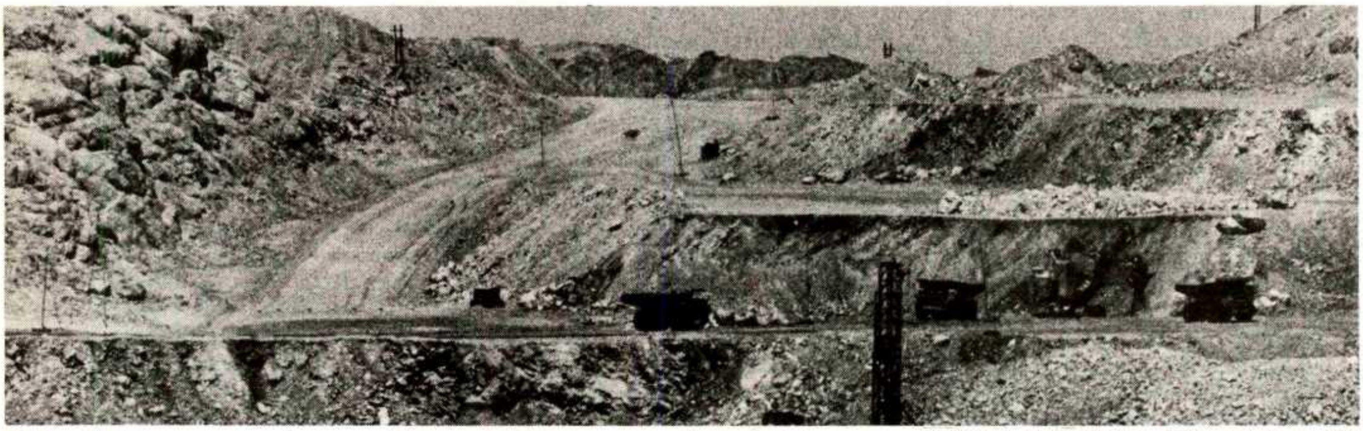

Uranium strip mining: "like going to the Moon"

Unhappy hunting grounds: the Indian end of nuclear energy

Joe Schwartz talks to Winona LaDuke and Herb Blatchford of the American Indian Environmental Council about the hazards of uranium mines in native lands

"THEY start by taking core drills. Then the rigs move in, next to a house or a corral, it doesn't matter to the companies. The pumps spray radon saturated water out on the ground where it forms radioactive pools. Animals drink it. Children play in it. The dogs become listless. The children sleep all night without resting. They complain of nausea. Dietary problems and gastro-intestinal complaints are common."

Herb Blatchford, 51, and Winona LaDuke, 20, of the American Indian Environmental Council (AIEC) were in London last week for a short stopover on their way to the Copenhagen Conference on Uranium Mining and Indigenous Cultures on 18-20 October. "It's an international conference of irradiated natives" says Winona LaDuke drily. But, with most of the world's uranium mining taking place on the lands of native people, Blatchford and LaDuke have a lot of first hand experience about what goes on at the "front end" of the nuclear fuel cycle. SCRAM, the Scottish coalition of environmental groups, had invited them to Edinburgh to discuss the planned uranium mine in the Orkneys that will fuel the Torness power plant (20 September, page 171).

"The disruption is unbelieveable" says Blatchford. "In fact it's so bad that Nixon's Project Independence in 1973 called for 'National Sacrifice Areas.' The Dineh (Navajo) lands in the New Mexico and Arizona were to form the southern end of an energy corridor extending up through Wyoming, South Dakota and into Saskatchewan that would end up a total wasteland.

"The largest uranium strip mine in the world is located at Laguna Pueblo near Albuquerque. It's owned by Anaconda. Going there is like going to the moon. People are listless, the animals show no motion, there are machines grinding all the time. The mine covers the whole valley and the Rio Paguate is a fluorescent green. Originally it was farming community."

Energy development in the American Southwest is intense. Navajo lands at present house four coal mines, five coal fired power plants, 36 operating uranium mines, six operating uranium mills and six abandoned uranium mills. Winona LaDuke describes how these arrangements are negotiated. "The leases are negotiated through the Bureau of Indian Affairs in consultation with tribal councils. The tribal councils are a creation of the BIA, the usual form of native government being a council of elders with decisions taken by consensus. The tribal councils are funded by the BIA. In the Navajo lands there are 74 members of council earning $13,000 a year. The average Navajo income is $1,400 a year.

"Royalties are minimal. The present rate of $0.6 a pound for yellowcake is a royalty of 1.2% of the market price of $50 per pound. You have to compare this to the standard royalty of 12% paid to the Bureau of Land Management and the Federal Parks for resouces taken out of other federal land. The royalty is paid to the Area Director of the BIA and is given out only with the approval of the Secretary of the Interior. We are legally wards of the government not beneficiaries so we have to beg back the money that is supposed to be ours."

Court cases to change the legal status of the Indian nations or to alter present contracts have failed. Last spring the Jicarilla (Apache) in Northern New Mexico managed to win a tax case meaning that they will no longer have to pay federal tax on their royalties. LaDuke says this is unique to native communities in the world. "There's nothing compared to it. It's a system of involuntary servitude. For example, in one year the Dineh (Navajo) nation exports enough energy to provide the needs of the state of New Mexico for 32 years. But 85% of Navajo households don't have electricity. This makes the Navajo nation an energy colony."

But finally, it is the hazards of uranium mining itself that is the urgent issue. "Eighty five per cent of the released radioactivity remains in the tailings. Rainfall tends to release the Rn222 which in New Mexico rises to the Continental Divide and then settles back down collecting in basins. We are starting to see long range effects now."

After a major exposé in the *Washington Post* last year, the US Public Health Service attempted to locate some 250 Navajo and 400 white miners who had worked the Red Rock Cove mine near Shiprock operated by Kerr-McGee. Of 100 miners eventually surveyed, 25 had died while still in their 40s and an additional 20 were found to have cancer.

The environmental prognosis for non-miners appears to be poor. A Los Alamos report (*Uranium Mill Tailings, Environmental Implications,* February, 1978) was pessimistic about the possibilities of controlling radon release. "Our research indicates that 12 feet of clay are required to reduce the radon exhalation rate by 99% and the remaining 1% is still about four times the typical soil exhalation rate.

"Perhaps the solution to the radon problem is to zone the land into uranium mining and milling districts so as to forbid human habitation."

In spite of the demonstrable hazards and what Herb Blatchford calls the "ultraexploitation" of native peoples there is going to be opposition to change. The AIEC called a demonstration at Mt Taylor to protest at a Gulf Oil uranium mine and mill complex which plans to produce 100 million pounds of uranium. On the same day the Energy Association of Taxpayers, an organisation funded by the energy companies, sponsored an Energy Fair in nearby Grants, the self proclaimed "Energy Capital of the World." Following

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a parade with floats carrying the slogans "Who Sabatoged Three Mile Island?" and "Uranium for Our Children", New Mexico Senator Harrison Schmidt told the rally:

"I will do everything in my legislative power to eliminate the need for environmental impact statements for future uranium mines and mills." □

And in the meantime, in the UK, the Central Electricity Generating Board made a £150 million bid for a share in the Ranger uranium project on native lands in Northern Territory in Australia. According to the *Guardian*, the CEGB bid is to safeguard Britain's growing need for uranium which is expected to incrase by 2½ times in the next 10 years. □

Spanish scientists protest over looming cash crisis

Fifteen Spanish scientists last month published an open letter to their government. They claimed that research would be brought to a halt in 1980 if the government did not act quickly to provide funds. The letter, which appears below, was communicated to us by a Barcelona scientist, Dr Juan Subirana, whose further comments appear this week in correspondence (p 424). The scientists' difficulties appear against an annual inflation rate of 25%, an extensive attempt to curb government expenditure, and a near-complete lack of scientific representation in parliament

“State-financed research groups in Spain are in great difficulties for lack money — and if the government fails to take immediate action, their problems will be dramatically worse next year. On 31 December this year financial support for all projects approved by the Comision Asesora de Investigacion Cientifica y Tecnica will end. The projects were announced in 1975 and 1976.

As you know, scientific and technical research in this country has three financial sources:

- Institutional finance through the universities, the council for scientific research (CSIC), and the research centres of ministries of agriculture, industry, health, public works, transport, and communications;
- Grants from Spanish or foreign foundations and those derived from bilaterial international agreements; and
- The Comision Asesora de Investigacion Cientifica y Tecnica (CAICT).

The first of these sources, instutitional finance, covers little more than maintenance, particularly in the case of the universities and the CSIC. Research funds come mostly from foundation and international grants and the CAICT. In the universities and the research centres of the CSIC at least 80% of the funds available for research come from the CAICT. Yet up to this date there has been no invitation from the Comision for proposals for new projects beyond the end of this year.

Since there are no substantial alternative sources, research — at least in the universities and the CSIC — will virtually cease on 1 January 1980.

The consequences are obvious: projects underway will have to be abandoned, in many cases with the loss of everything already achieved; and scientists in training and contracted auxiliary personnel will have to leave their laboratories.

The Comision Asesora was established in 1958. Recently it has come to belong to the Ministry of Universities and Research, with its annual budget of 1.1 billion pesetas ($17 million) dedicated to research. We understand that the Comision is being reorganised, but we do not understand why this reorganisation has halted the existing mechanisms for distributing Comision funds. We also understand that the government has prepared a three-year plan for research support which will be discussed in parliament, but it seems clear that no funds will be forthcoming from that plan until the second half of next year.

For the survival of science, we ask the government, through the Ministry of Universities and Research, to take urgent measures. We scientists signing below believe we speak as representatives of many others when we propose that the government should:

- Immediately announce the offer of Comision Asesora research grants for 1980, and assure that the selection procedure is complete by the end of the year
- As a matter or urgency, put the three-year plan for research before parliament.

So our country may have a minimum of independence and intellectual respect, political changes must go along with deep changes in our scientific and technological policy; but these changes must not be made at the expense of a temporary paralysation of Spanish research.”

Signed: Manuel Ballester, CSIC Barcelona, Nicolas Cabrera, Universidad Autonoma Madrid, Antonio Cordoba, Universidad Complutense, Jose Maria Fontbote, Universidad de Barcelona, Alberto Galindo, Universidad Complutense Madrid, Antonio Garcia Bellido, CSIC Madrid, Antonio Garcia Olmendia, Universidad Politecnica Madrid, Antonio Gonzalez Gonzalez, Universidad de la Laguna, Jose Antonio Jimenez Salas, Universidad Politecnica Madrid, Jose Maria Jover, Universidad Complutense Madrid, Ramon Margalef, Universidad de Barcelona, Francisco Rodriguez Adrados, Universidad Complutense Madrid, Jose Maria Serratosa, CSIC Madrid, Alberto Sols, Universidad Autonoma Madrid, Agustin Udias, Universidad Complutense Madrid.

The day after this letter appeared in the Spanish press, a meeting of the joint council of the CSIC, the universities, and the Ministry of Universities and Research took place. The meeting had already been planned as part of the efforts to create a research policy for Spain. The press commented on 28 September:

El Pais (Madrid)

"The Ministry of Universities and Research is studying the reorganisation of the CSIC as one of the first steps in the redefinition of research supported by the administration. Among other measures there are the preparation of a general report on research in Spain, the three-year (1980-82) plan of research, cooperation with other administrative bodies, etc. In particular the remodelling of the Comision Asesora de Investigacion Cientifica y Tecnica is also being considered.

King Juan Carlos: trouble with science

"Publication appears imminent in the *Boletin Oficial del Estado* (the official government journal) of a decree that will approve the new bye-laws of the Comision, which is the advisory board of the government in scientific policy, and which has as a main mission the approval of research projects under government support and the distribution of funds to finance them."

La Vanguardia (Barcelona)

"[The Comision Asesora has decided] not publicise now an annual announcement of projects, as requested by a group of scientists in a recent open letter to the Ministry of Universities and Research. The reason is that the 250 million pesetas ($4 million) available to the Comision would not solve the scientists' economic problems, while it seems more convenient to transfer this quantity to the next year and not to proceed with the announcement before January 1980".

It thus appears that the situation of science in Spain is far from clear, and is continuing to give scientists considerable anxiety. □

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West Germany: a nuclear incident every three days

OVER the past two years, one incident or accident has occurred on average every three days in West German nuclear power stations, according to a recent report of the Bundesverband Bürgerinitiativen Umweltschutz e.v. (BBU), the chief organisation of German environment protection groups and associations. The report, "Accidents in German Nuclear Power Stations. Publication of Confidential Incident Reports of the Federal Government", also claims that most of these incidents have been kept secret. Information for the report was obtained from the files of the Gesellschaft für Reaktorsicherheit (GRS), the German Reactor Safety Society.

Publication of the BBU report came shortly after the German Minister for Research and Technology, Volker Hauff, released part A of the report of a risk study of nuclear power stations in West Germany — the German version of the Rasmussen Report. The study concluded that the probability of an accident is extremely low. Ironically, the author of the study is the GRS, the very same society from which the BBU obtained its information.

GRS is a limited liability company based in Cologne with the Federal government, Bavaria, Northrhine-Westphalia, some technical supervisory bodies and the German Lloyd (an insurance company) as shareholders. In 1974/75, the Federal Ministry of the Interior commissioned the GRS to collect and categorise all data relevant to incidents and accidents in German nuclear power stations (and also, as far as possible, in foreign ones). The aim was to improve safety by mutual exchange of information between utilities and concerned authorities.

In fulfilling this commission, the GRS obtains short standardised reports from the utilities which have been prepared whenever deviations from routine operations have occurred. The deviations or incidents (*besondere Vorkommnisse*) are divided into three categories according to their significance for the safety of the plant and the public:

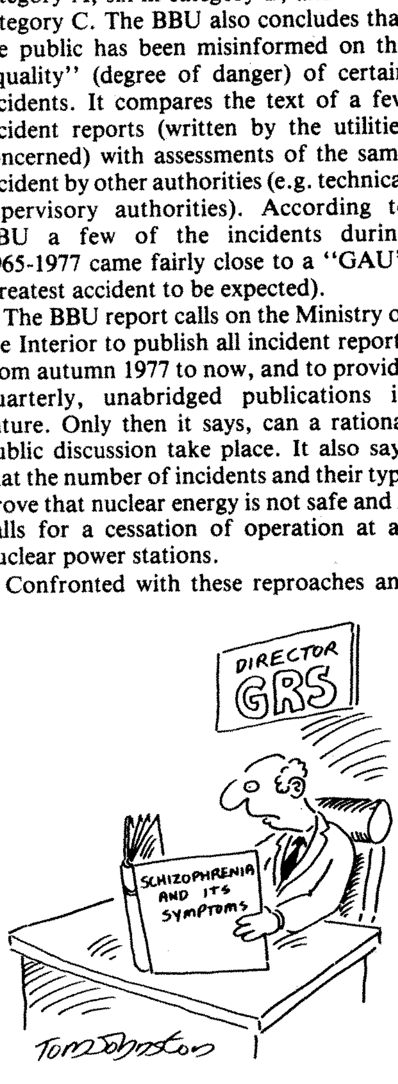
- Category A includes incidents that immediately threaten safety, for example: emission of more than the maximum permissible amount of radioactivity; breakdown within the primary circuit combined with emission of radioactivity or with coolant leakage.
- Category B includes incidents that are a potential threat to safety, for example: unplanned emission of radioactivity below permitted amounts; breakdown of system components with importance for safety during tests (if not during normal operations).
- Category C includes any other kind of deviation from specified norms and safety regulations.

The utilities themselves categorise individual incidents so that the GRS simply list the collected data and sends both the incident lists and the individual incident reports four times a year to the Ministry of the Interior, and its other shareholders.

It was these lists and reports from 1965 to autumn 1977, that the BBU has published together with a few comments, interpretations and supplements from other sources. Its report is not easy for the uninitiated to understand, because no systematic interpretation has been done, incidents of differing relevance being catalogued only by time and location. But it is a unique source of information in West Germany. In the introduction, the BBU compares the official risk study report with the confidential lists and incident reports. It concludes that the public has been misinformed on the number of incidents which have occurred. For example in 1976 according to GRS data 139 incidents, 24 in category A, 85 in category B, and 30 in category C occurred. A Federal Government document, however, called "On the peaceful use of nuclear energy" mentions only 14 incidents in 1976, six in category A, six in category B, and two in category C. The BBU also concludes that the public has been misinformed on the "quality" (degree of danger) of certain incidents. It compares the text of a few incident reports (written by the utilities concerned) with assessments of the same incident by other authorities (e.g. technical supervisory authorities). According to BBU a few of the incidents during 1965-1977 came fairly close to a "GAU" (greatest accident to be expected).

The BBU report calls on the Ministry of the Interior to publish all incident reports from autumn 1977 to now, and to provide quarterly, unabridged publications in future. Only then it says, can a rational public discussion take place. It also says that the number of incidents and their type prove that nuclear energy is not safe and it calls for a cessation of operation at all nuclear power stations.

Confronted with these reproaches and demands, the Federal Ministry of the Interior reacted almost too calmly. It said that the Bund and Länder have been informing the public of all relevant incidents in German nuclear power stations since 1966 and that Parliament is continually informed of all incidents (not only those with safety relevance). Public information is given only on incidents that threaten the safety of a power station and/or the public according to "expert assessment". The 14 incidents, mentioned in the Federal Government's documentation for 1976 are according to the ministry, "representative" and not the total number of incidents.



According to competent experts within the Federal Ministry of the Interior, there is confusion over nuclear information in the FRG revealing a lack of competence and a lack of political awareness that the public must be totally and regularly informed. There is the unique situation in the FRG, that the utilities possess a kind of "copyright" over incidents. This stems from the "Gewerbeordnung" (trade and manufacturing ordinance) a law made for small manufacturers and the "Atomgesetz" (law on nuclear energy). Both are influenced by the same ideas on 'rights of possession'. There is, therefore, no absolute compulsion on the Bund or Länder to publish information on incidents, except where it serves to improve safety conditions (as stated in the "Atomgesetz").

The prime responsibility for nuclear incidents, however, rests with the Länder. The Federal Government, represented by the Ministry of the Interior has only certain supervisory rights over the relevant Länder ministries although it has to inform the Bundestag. Now the Länder provide information on each incident. This used to be done by the Federal Ministry of the Interior before 1976/77, when a first list of incidents (compiled originally for the competent committee of the Bundestag) was published in the ministry's journal *Umwelt* (environment). The list, however, was not complete and the individual incident reports were not published — possibly because of the utilities' "copyrights". As was explicitly said, the list was only "representative".

There now exists an extension to the list for 1977 and 1978. It went to the competent committee of the Bundestag at the end of May 1979 and will be published in *Umwelt* on 12 October. Is this chance or a consequence of the BBU publication? A spokesman for the ministry claimed that "publication was planned long ago, it had to be prepared before the summer recess". The ministry now says that there are no intentions of publishing quarterly or regular lists of incidents or of publishing the individual incident reports made by the utilities. **Klaus Höpfner**

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Germany to decentralise nuclear waste treatment

GERMANY'S ambitious plans to build a huge nuclear waste disposal centre at Gorleben in Lower Saxony have finally been dropped, despite pressure from the federal government. On 30 September Chancellor Helmut Schmidt and the Minister-President of the eleven German Länder agreed to abandon the plan after a series of lengthy negotiations. And the nuclear industry have now decided to approach the problem in smaller stages.

After an international six-day hearing last May (24 May, page 283), Minister-President Ernst Albrecht of Lower Saxony (CDU) had rejected plans to build an integrated nuclear waste disposal centre at Gorleben. However the Federal Government under Chancellor Schmidt (SPD) continued to adhere to the concept of an integrated waste disposal centre. The result was stalemate and additional delays to nuclear expansion in West Germany — because of the law-courts' insistence on feasible nuclear waste management before granting licences to new nuclear power plants.

But now Chancellor Schmidt and the Minister-Presidents of the Lander have agreed on the following points:

- West Germany is to manage the whole of its nuclear fuel cycle but in small units at different sites.
- No reprocessing plant of any kind is to be built at Gorleben in the foreseeable future. Instead, the possibility of building one or more small reprocessing plants at other sites in West Germany is to be investigated and the final decision on reprocessing is to be taken by the mid 1980s.
- Testing of Gorleben saltdomes for final storage will continue.
- Lower Saxony and Northrhine-Westphalia have agreed to build two or three intermediate storage facilities which will be needed for about 20 years before a final solution to the storage problem is developed. The Bavarian criticism that "compact storage facilities" within nuclear power stations "contribute to the intermediate storage problem" has been taken into account.
- Research into alternatives to reprocessing, in particular final storage of unreprocessed spent fuel, is to be supported.

But there many unknowns in this political solution. One certainty, however, is that no reprocessing plant will ever be built at Gorleben — Minister-President Ernst Albrecht is trying to avoid endangering his political career, especially as he is a potential candidate for the chancellorship in 1984 or 1988. A complete new reprocessing plant may never be built especially if ideas to enlarge the existing experimental plant at the nuclear research centre in Karlsruhe are adopted.

However the problems of siting intermediate storage facilities might be only marginally easier than those of siting reprocessing plants and final storage facilities. In Ahaus (Northrhine-Westphalia), for example, which has already been selected as the site for intermediate storage ponds, the CDU lost 20% of their former votes during local elections last week while a recently-formed opposition group won 25% of the votes.

Further, the question of whether the law-courts will accept the intermediate storage compromise as a solution to the waste management problem for the time being is still open. And last but not least, the congress of the governing SPD (Social Democratic Party) will be held in Berlin in December. Nuclear power will top the agenda.

Klaus Hopfner

Soviet psychiatrist attacks 'Snezhnevskiism'

MOSCOW'S psychiatric establishment has now produced its own internal dissent — according to US psychiatrist Walter Reich. He was referring to an article, soberly titled "Differentiating Exogenous Psychiatric Illness from Schizophrenia", which was apparently sent abroad by unofficial channels and appeared in the August issue of the US "Archives of General Psychiatry".

Of itself, the article appears to be simply a criticism of current Soviet diagnostic procedures. On the basis of an analysis of more than 300 patients, the author, Dr Etelii Filipovich Kazanets of Moscow's Servskii Institute of Forensic Psychiatry speaks of many "over-diagnoses", "incorrect diagnoses", the necessity for "revising many long-standing diagnoses" and the "criteria, associated with the Snezhnevskii school" which "over-extend the diagnosis of schizophrenia".

For Andrei Snezhnevskii, head of the Institute of Phychiatry of the Soviet Academy of Medical Sciences, all mental illness is some form of schizophrenia. Indeed, he has identified a number of types which seem of specifically Soviet provenance, including "sluggish" schizophrenia which, it appears, has no presenting symptoms except political dissent.

Dr Kazanets, it must be stressed, does not in any way refer to the "political" application of Snezhnevskii's theory — although coming from the Serbskii Institute which has been responsible for many diagnostic reports on dissidents. Indeed, he does not even say that the patients whom he reviewed were in fact in good mental health — only that they were suffering from brief "exogenous psychoses" which could have been cleared up in a short time, without for the long "rehabilitation" process with compulsory attendance at psychiatric outpatient clinics and domicilary visits.

Where, then, lies the "daring" which Dr Reich — known as a moderate and cautious commentator — finds in the article? It should be stressed, perhaps, that even apart from its provenance, the study is, in the words of Jack Weinberg, former president of the American Psychiatric Association, scientifically "significant".

Of recent years Snezhnevskii's theory of schizophrenia has made a number of western psychiatrists wonder how long a meaningful dialogue with their Soviet opposite numbers would be possible. Irrespective of the political misuse to which they could be put, Snezhnevskii's views, it was felt, could impose a Lysenko-type isolation on Soviet psychiatry.

To attack these entrenched ideas, Dr Kazanets was obliged to send his manuscript abroad "privately", bypassing the official postal (and censorship) channels — an action which in itself could have serious consequences. Yet the article is, in itself, controlled in tone. Certainly, as western commentators have been keen to point out, he notes that the "prolonged retention of individuals on psychiatric, dispensary lists long after their recovery infringes on rights" (*not* "human rights" as it has been misquoted). He also implies, however, that Soviet society as a whole may suffer: the "illegal inclusion of persons not mentally ill on psychiatric rosters" may entail "criminal elements" being unjustifiably freed from "responsibility for their crimes".

Dr Kazanets has long been considered something of a "loner" and "troublemaker" by his colleagues, and it is not surprising that he should be the first to raise his voice in qualified criticism of Snezhnevskii. Nor is it entirely surprising, that some cracks are occuring in the monolith of Snezhnevskiism. Already some "establishment" psychiatrists are known to have associated themselves with the dissident "Working Commission for Monitoring the Use of Psychiatry for Political Purposes" (a body which in spite of official harassments is still producing a regular bimonthly *samizdat* bulletin).

One should not, however, be over-optimistic. So far there is little indication that Dr Kazanets has any great following, although at least one of his colleagues has been prepared, privately, to testify to his intelligence and "stimulating" personality. Such an assessment may well be needed in months to come — for already there are hints that certain Soviet "establishment" psychiatrists are referring to Dr Kazanets's challenge as itself as symptom of — "schizophrenia".

Vera Rich

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news in brief

US Senate agrees to circumvent environmental legislation: The US Senate agreed last week to support President Carter's proposals to establish an Energy Mobilisation Board to cut red tape on selected large energy projects. This was despite the objections of environmentalists and state and local groups that the board's powers to speed up the review process could undermine many of the environmental laws that have been passed in recent years, and would trample on the rights of local governments. An intense lobbying effort by Carter and by the oil industry managed to secure the defeat of an amendment from Senator Abraham Ribicoff and Senator Edmund Muskie that would have eliminated these provisions from the Bill setting up the board. The measure now passes to the House of Representatives, which is already considering two versions of the Bill, one of which — passed by the House Commerce Committee three weeks ago — would give the board even stronger powers to ignore what it considered to be unreasonable environmental legislation. Meanwhile, a report prepared by a Senate budget committee and issued last week stated that the President's proposal to reduce oil imports through massive development of synthetic fuel production cannot presently be justified on economic grounds.

Fuelling guided missiles can seriously damage your health, as well as causing a major threat to the environment, according to Chinese military experts. Charging a missile with liquid fuel is a delicate task, involving an accurate determination of the temperature in the fuelling trough. Conventional sampling methods entail drawing off a sample of up to 40 kg. This deteriorates in contact with the air during the measuring process, and is afterwards voided, seeping into the soil and polluting the ground-waters. There is also considerable atmospheric pollution and health hazard, apparently from reaction fumes. To overcome these disadvantages (and to save the state the 1500 yuan spent annually on the wasted fuel), two members of the "fuelling squadron" — platoon leader Wang Enchen and technician Xun Chengzhi — have developed a closed-loop test method which gives a direct readout from a temperature gauge permanently fixed inside the test chamber. In a recent broadcast, Peking radio stressed the prolonged research in metallurgy and the science of refractories needed before on effective system was perfected. But now, said the commentator, this sophisticated system has saved state research and development funds, improved working conditions for the fuelling personnel, and shortened the average fuelling time from ten hours to six (and on one occasion, only 2.5).

US experiments carried by Soviet satellite: An international collaboration on the effects of weightlessness on animal and bacterial growth was launched 26 September in the Soviet Union. The major payload consists of 38 white rats and 60 fertilised Japanese quail eggs shared among experimenters from the participating countries which include the US, France and Czechoslovakia. The launch is similar to two previous Cosmos missions in 1975 and 1977 in which the US also participated. In addition to experiments on embryonic growth in both mammals and birds, studies will be made of changes in animal muscle and bone in an attempt to investigate the reasons for calcium loss in the bones and loss of muscle strength by astronauts and cosmonauts during prolonged space flights. The flight will last three weeks.

West German government outlines biotechnology programme: In response to a formal parliamentary question, the Federal Ministry for Research and Technology (BMFT) has published its appraisal of West Germany's present position in biotechnology. West Germany is in the middle rank for biotechnological research, well behind the US and Japan but with a research effort comparable to the UK and France, says the BMFT. In certain sectors, West Germany has reached international standards — for example in the production of single cell proteins from methanol, in cell culture technology, in basic research for bioreactor development, and in the production of compounds by enzymatic conversion.

For the first time the 1980 research budget contains a separate entry for research and development in biotechnology, amounting to 37 million DM. The government's aim is to improve the current standard of research and to facilitate industrial implementation of research results. The government also wants to reach international standards in the following areas: design and construction of bioreactor systems in sewage treatment; biosynthetic processes; fixation of bio catalysts; gene technology and plasmid research; biological nitrogen fixation; and bioenergetics and renewable raw materials.

French government backs down on faulty nuclear start up: A five-day strike by the socialist and communist trade union federations, the CFDT and CGT, has forced the French government to postpone the loading of its Gravelines 1 and Tricastin 1 nuclear reactors. The French electricity authority will convene health and safety committees to investigate the implication of the fissures in the tubular plates of the heat exchanger (*Nature* 27 September) and will make further checks, not expected to take more than three weeks, on the faulty parts. The existence of the cracks up to 6 millimetres deep was made public on 22 September but the government announced its plans to go ahead with the fuelling on 3 October. Although up to 20 reactors are affected, the government argued that the cracks would have to be 60 millimetres deep to constitute a serious hazard. The government's decision provoked a storm of criticism throughout France as political and environmental groups joined the trade unions in protesting the government's decision. Eighteen organisations issued a joint statement on 3 October calling on the government to "refuse this fantastic industrial gamble which could have very heavy consequences." The faults in the heat exchanger plates raise the question whether the plates could actually break. Collapse of the plates means instant and uncontrollable release of water from the primary cooling circuit which would result in a complete loss of coolant.

WHO report criticises current European occupational health programmes: The World Health Organisation has released a stinging critique of routine health examinations for workers exposed to occupational hazards. The report says that European countries have placed too much emphasis on medical examinations and not enough on elimination of industrial hazards. "The fact is sometimes overlooked that the examination of a worker does nothing to eliminate the hazard . . . . Technical improvements should be considered first and the goal should be to make examinations unnecessary by eliminating hazards in industries." The study, a survey of industrial health practices in Finland, the German Democratic Republic, Poland, the USSR and the UK, questions the efficacy of periodic health examinations which are undertaken on a "considerable" scale by European governments. Criticising the lack of knowledge of physicians of the physical and mental requirements of specific jobs, the difficulty of predicting the consequences to health of exposure to industrial hazards and false negative results of many clinical and laboratory tests, the report claims that present practices are wasteful and avoid the real problem of prevention. "If the cost and benefits of the examinations are compared with the really effective alternative, the elimination of the hazards, the present practice in many countries will be shown to be uneconomic."

● The second author of last week's article on geological aspects of nuclear waste disposal (4 October, 332-4) should have been D.A. Gray, not D.A. Greenwood.

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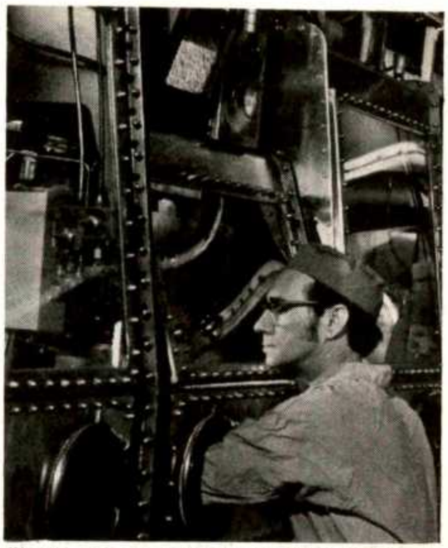
Containing Recombinant DNA: how to reduce the risk of escape

David R Lincoln, Laurence R Landis and **H Andrew Gray** describe a 'fault tree' analysis of containment facilities which, when applied to Fort Detrick (right), enabled them to suggest how to cut the chance of an escape from one in 15 years to one in 22. The authors are from Carnegie-Mellon University, Pittsburgh



The technique of recombining DNA from a foreign source into a host organism could have harmful consequences only after a sequences of events, if at all. An organism must escape from the containment facility, there must be another living system present, the living system must become infected, and the recombinant DNA (RDNA) organisms must bear some harmful action which is released to the living system. (This very generalised framework does not account specifically for differences in the action of toxic compounds, immune responses, or other possible responses.)

Several biological studies have been performed during the last six years which have influenced the understanding of the probability of infecting a living system, and of the infection having harmful effects.[1-5] However, limited research[6-8] has been performed to increase the knowledge of the adequacy of capabilities of the different physical containment facilities for RDNA experiments, or for experiments with known pathogenic organisms and viruses. We describe a framework for assessing the containment system reliability with fault tree analysis.

Engineers use fault tree analysis to indentify the various combinations and sequences of component failures which lead to an undesirable event in a system — such as the melt down of a nuclear reactor. The technique is particularly suited to the analysis of complex systems because it can effectively model a vast number of system components.

The procedure is performed stepwise, with the first decision being a choice of the 'top' event, which is an undesirable event associated with the system. The next step is to determine all the necessary conditions, or secondary events, which will cause this top event. If two secondary events must both occur to cause the top event, these two events will be linked in the tree to the top event by an 'and' gate. However, if either of the events will cause the top event, the secondary events will be linked to the top event by an 'or' gate. An ordering of causative events will be generated through subsequent steps, until finally a set of primal events is reached. These are events whose causes need not or cannot be determined.

'Cut sets', which are independent paths or sets of primal events which cause the top event, are then determined. Any cut set having no subsets indentical to any other cut set is defined as a 'minimal' cut set. The frequencies and probabilities of the minimal cut sets are calculated, and the frequency of the top event is the sum of the frequencies of all the minimal cut sets. A more detailed discussion of the procedure and method of calculation has been published previously.[9-10]

Our study focused on one P4 containment facility, Building 550 of the Frederick Cancer Research Centre (FCRC) at Fort Detrick, Frederick, Maryland. A P4 laboratory is the most stringent containment facility for DNA research. In particular, this study examined the escape routes from Rooms 214 and 215, which contain the Class III cabinet areas of the P4 facility. The facility is undergoing some structural changes, and we obtained detailed architectural drawings of the reconstructed building with the cooperation of the engineering staff at FCRC. The drawings included floor plans, ventilation and piping diagrams, and details of the electrical systems.

The FCRC 'Safety and operations Manual' and FCRC personnel themselves, verified procedures for entering an exiting the facility, experiments, and other operations of the facility. Fig.1 shows a schematic which was developed from the architectural drawings of the air ventilation and liquid modes of transport of materials from Rooms 214 and 215.

Air is supplied to Rooms 214 and 215 from 'clean' areas of the building by fans, creating a pressure differential between these two areas and producing a directional air flow. Supply air is passed through two filters, one low efficiency filter for large particles and one 95% efficient filter. An air pressure difference is also maintained between the room air and the Class III cabinets. Air is exhausted from the rooms and the cabinets through the filters indicated in the diagram.

The sole water inlet to Rooms 214 and 215 is into the Class III cabinets. Before the water enters the facility, it passes through a back flow preventer. No drinking water is available in the rooms, and building drinking water is supplied by a separate line which is tapped off the main line before the water reaches the back flow preventer.

There is one floor drain in each room and several cabinet water drains. Each drain has a minimum six-inch trap and all liquid effluents drain to a common pipe. The liquids are treated by the Fort Detrick Sewage Sterilization Plant, and then by a municipal sewage treatment plant having primary and secondary treatment.

Two additional modes of organism escape were considered after an examination of the facility: contact modes, and natural catastrophes. The contact mode included the human contacts through ingestion, injection into the blood, or transport on skin or clothes, as well as organism contact and escape on any

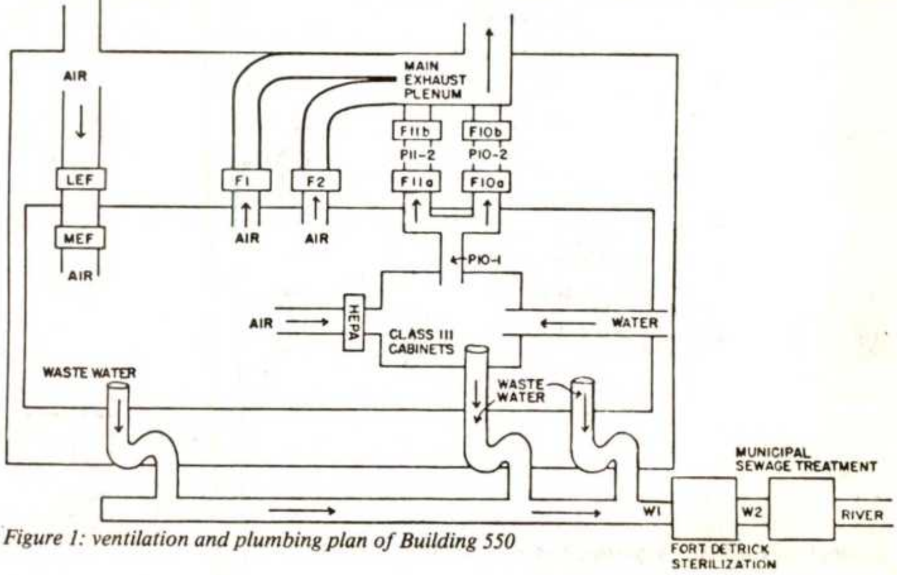


Figure 1: ventilation and plumbing plan of Building 550

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materials (instruments, glassware, paper, laundry, media, cultures, etc.) which are transported from the facility.

Certain natural catastrophes could occur during the lifetime of the facility which could reduce or eliminate the effectiveness of all containment carriers. These could include earthquakes, major storms and fires. Although the probability of these events may be remote, the probability of escape given the events is very high. Therefore, these escape routes were also considered.

The top event in this fault tree was considered to be "appreciable number of RDNA organisms escape" from the P4 facility. It is recognised that different numbers of organisms would be used in different experimental protocols. In addition, aerosol generation would be likely to be a function of the experimenters' capabilities.[6,7] The survivability of organisms in different media (air, water, solid wastes) would also play a role in determining the likelihood of escape of a given number of organisms. The transit time to the exterior of the facility was considered to be very low with respect to organism survival time.[7] These factors were not considered explicitly in this first application of fault tree analysis to recombinant DNA containment facilities. It should be noted however, that more detailed studies to develop appropriate models and experimental data could lead to an incorporation of these variables in a second generation tree.

The four major escape routes are shown in the fault tree in Figure 2:

- air ventilation transport,
- liquid waste transport,
- contact transport, and
- major catastrophes.

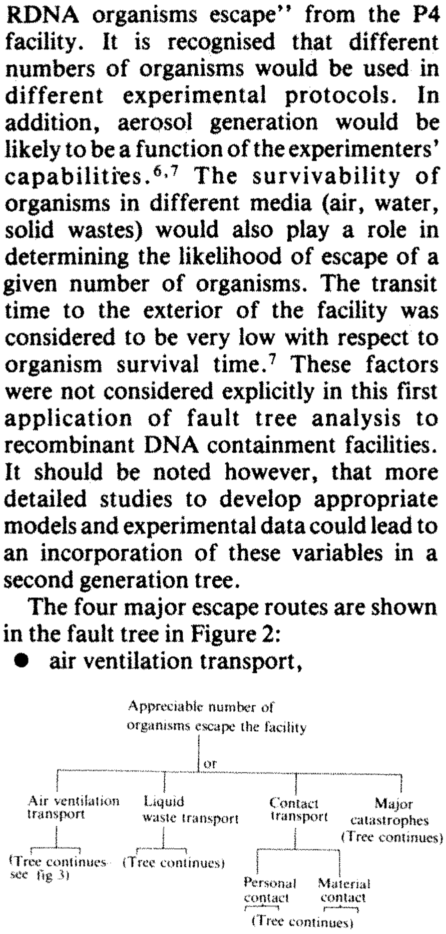

Figure 2: fault tree showing major escape routes

The events leading to air ventilation transport are shown in Fig 3. The primal events (57 in the whole tree) can be found at the bottom of the tree. Details of the complete tree are available from the authors.)

For each primal event there is an associated frequency of occurence, λ, which is the inverse of the mean time interval, in which the event will occur exactly once. The mean time to repair, τ, in the context of fault tree analysis, is the time needed either to repair the fault or to move the system to a safe state. For some primal events, it is easier to assign an unavailability, q, instead of λ, and τ. Unavailability is the probability that a component is not available.

The components of the P4 laboratory have been operational since April 1977, which was an insufficient time period for developing a useful data base of operations. However, similar component designs exist in other industrial applications, and estimates for q, λ and τ were developed after a discussion of the primal events in the fault tree with G.J. Powers, Department of Chemical Engineering, Carnegie-Mellon University. A list of the values used in the base case fault tree is available from the authors. It should be recognised that these values can be updated with experimental data as they become available.

The minimal cut sets were developed from the fault tree by applying the algorithm developed by Powers[10], revealing over 200 potential escape routes. The frequency of each cut set was calculated using our estimates of q, λ and τ with the frequency equations of Powers.[10] In order to obtain the freqency of the top event, the frequencies of the minimal cut sets were summed. The mean frequency of the top event 'escape of an apppreciable number of organisms, was calculated to be once in 15 years.

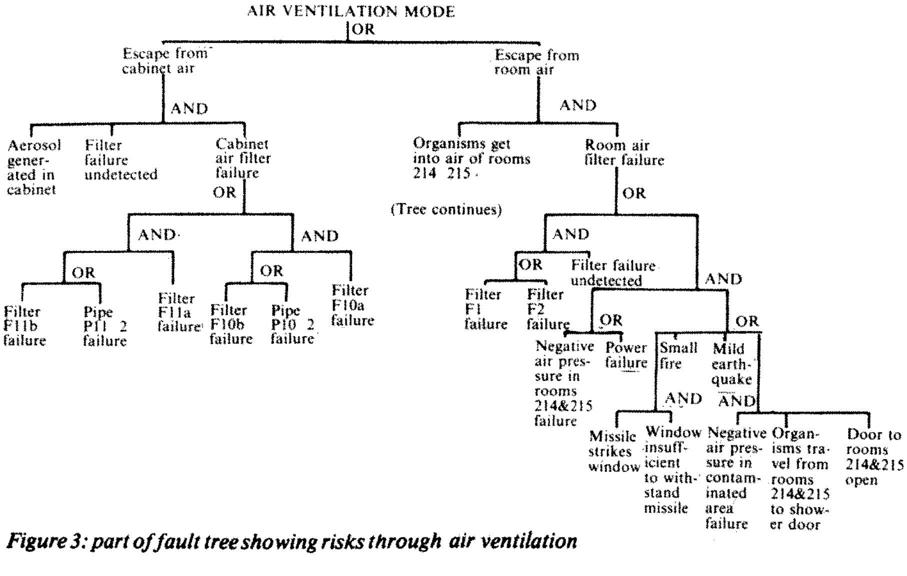
AIR VENTILATION MODE
OR
Escape from cabinet air
Escape from room air
AND
AND
Aerosol generated in cabinet
Filter failure undetected
Cabinet air filter failure
OR
Organisms get into air of rooms 214 215
(Tree continues)
Room air filter failure
OR
AND
AND
AND
OR
OR
Filter F11b failure
Pipe P11 2 failure
Filter F11a failure
Filter F10b failure
Pipe P10 2 failure
Filter F10a failure
OR
Filter failure undetected
Filter F1 failure
Filter F2 failure
AND
OR
OR
Negative air pressure in rooms 214&215 failure
Power failure
Small fire
Mild earthquake
AND
AND
Missile strikes window
Window insufficient to withstand missile
Negative air pressure in contaminated area failure
Organisms travel from rooms 214&215 to shower door
Door to rooms 214&215 open

Figure 3: part of fault tree showing risks through air ventilation

The "importance" of a minimal cut set in a fault tree is defined as the frequency of that minimal cut set divided by the frequency of the top event.[10] This may be interpreted as the probability that the events of a minimal cut set have occurred, given that the top event has occurred. From Table 1, it can be seen that approximately 75% of the escape potential can be attributed to three minimal cut sets. With the top eight cut sets, approximately 95% of the escape potential is accounted for.

Table 1: CUT SET IMPORTANCE

Cut Set	Importance to Escape Frequency
Organism injected into blood	0.383
Filter F11a and F11b failure; filter failure undetected	0.191
Transport container leaks	0.077
Organism inhaled in Rooms 214 and 215; pipe P10-1 failure	0.046
Filter F10a failure; pipe P10-2 failure; filter failure undetected	0.025
Filter F11a failure; pipe P11-2 failure; filter failure undetected	0.025
Appreciable number of organisms in building; major storm	0.023

Table 2: PRIMAL EVENT IMPORTANCE

Event	Importance to Escape Frequency
Filter failure undetected	.473
Organism injected into blood	.383
Filter F10a failure	.217
Filter F11a failure	.217
Filter F10b failure	.191
Filter F11b failure	.191
Transport container leaks	.077
Aerosol generated	.061

The "importance" of a primal event is defined as the sum of importances of every minimal cut set in which the primal event is an element. [10]The potential "weak" links in the containment system are further indentified in Table 2. Of the top six primal events in importance, five involve the high efficiency particulate air (HEPA) filters F10a, F10b, F11a, F11b, F11b, which are located in the exhaust stream of the Class III cabinets. In addition, the event of injecting the organisms into the experimenter's blood by accident is also a significant contributor to the potential for organism release.

The frequency of escape can be modified by changing system and procedural variables. We tried modifications of the parameters of all primal events that appeared in both the set and primal event importance tables (tables 1 and 2). In particular, it was found that changes in the HEPA filter parameters will significantly modify the escape frequency.

In the base case, the mean escape

The authors would like to acknowledge the cooperation of Drs W Emmett Barkley and John Nutter, National Institutes of Health, Washington, DC and the assistance of Orley Bourhard, Everett Hanel, Stanley Nagel and Irv Swope at the Frederick Cancer Research Center.

frequency is once in 15 years, assuming a mean HEPA filter failure rate once every four years. However, if this is decreased to once in eight years, the mean escape frequency decreases to once in 22 years. On the other hand, if the filter failure rate increases to once in two years, the mean escape frequency increases to once in seven years. If the filter mean time to failure detection is reduced from six months to 1.5 months, then the mean escape frequency again declines to once in 22 years. The impacts of other system modifications can be assessed in a similar manner.

We recognise that our fault tree — part of which appears in figures 3 and 4 — does not describe all the system parameters and modifications which may be of interest in a more complete description of escape likelihood from the P4 system. In particular, there remain considerations of:

- a more explicit description of the dependency of the likelihood of escape on the amount of released material, possible concerns that the organism survivability, and therefore potential for harmful impact, may depend on the mode of escape,
- the effect of alternative experimental protocols on the escape risk, and
- inclusion of experimental data on λ, τ, and q for system components. In addition, further examination of the fault tree for common mode failures would be useful. It is intended that future studies will reduce some of these presently perceived deficiencies.

This study does not describe whether our calculated escape frequencies pose a significant risk. The probability of a harmful consequence is the product of several probabilities involving biological details of the recombinant organisms and their escape probability. Further studies would be needed in order to determine whether or not a P4 laboratory provides an "acceptable" risk. □

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Full steam ahead to save Kenya's fuel

Rift Valley gushers, courtesy of the Kenya Power Company

HARD-pressed by the rising price of oil and a growing shortage of fuelwood, Kenya, like many developing countries, badly needs alternative sources of energy. Fortunately, it has at least one: a huge natural reservoir of boiling hot water some 1,000 metres beneath the Rift Valley about 80 kilometres from Nairobi.

Interest in the area as a possible site for a geothermal power station began in the 1950s, but detailed exploration (financed by the United Nations Development Programme) did not begin until 1970 when four wells were drilled in a wild, semi-arid area known as Olkaria, about five kilometres south of Lake Naivasha. Eleven wells have now been drilled there, and a contract was recently placed for construction of the first 15 MW power station which will feed into the national grid by mid-1981.

The Olkaria Power Station will be the first full-scale geothermal power station in Africa (a 220-KW plant was installed in Zaire at a metal mine in the 1950s, but was costly and inefficient and was closed down with the mine). When Olkaria goes into operation, it is expected to supply about 5% of Kenya's electrical power. Another 15MW generator is planned for operation in late 1982, and the station's capacity will be expanded to reach 90 MW in the next 10 years. Total energy available from the reservoir has been estimated at 170 MW for 25 years.

Kenya has no known fossil fuel resources, and the rising cost of imported oil recently prompted the President, Daniel Arap Moi, to call for reduced consumption. But as T.S. Tuschak, a Canadian energy consultant currently on loan to the Kenya government through the UNDP, pointed out recently (July 5, page 2) savings in the essential sectors can at best be 7%, and consumption by private transport can only be reduced by about 10%.

Kenya's economy is divided into two sectors: the industrialised and the rural. The industrialised sector gets about 85% of its total energy supply from oil, the rest from hydroelectricity. The rural economy's energy source is almost 100% wood or charcoal. And while Kenya has not suffered as much as some countries from deforestation, a fuelwood shortage is likely and has already occurred in some areas. Population is increasing at 3.9% a year, causing not only increased wood consumption but declining land availability. Fuelwood consumption is currently 20 million tons annually.

The government's forestry department has introduced conservation and reafforestation programmes and has shown some interest in agroforestry, and public attention has been drawn by various groups to the advantages of tree-planting. But it is not possible to wean the mass of the population away from its reliance on wood as its principal source of energy.

Kenya's unused hydro-electric power potential is 600-700 MW. Total electricity demand, which has grown at 8-9% per annum and is expected to continue at that rate for the balance of the century, should reach 1200 MW by AD 2000. This would call for 1500-1600 MW installed capacity. Development of all the HEP potential together with the present installed capacity of about 400 MW would leave a gap of 400-500 MW. The total geothermal resource is believed to be between 170-500 MW which could fill that gap.

Kenya has so far evolved no rural energy policy. While there are arguments for and against rural electrification schemes in developing countries, Mr Tuschak believes that an increase in electrical consumption in certain areas of Kenya could reduce its growing consumption of wood.

"In my estimation there are about 1-1.5 million people in the rural sector who are better off in financial terms and can afford to pay for commercial energy, or at least part of the cost," he told *Nature*. "Many of these people have already requested electricity and others would be happy to have it. If they are supplied with electricity they will stop consuming wood, and Kenya could perhaps cut down wood consumption by 10% or more".

Although hydro stations provide most of Kenya's electrical energy at present, hydro generation is expensive to run because the country's rivers have highly variable seasonal flow. Capital costs are also high. Under such conditions, geothermal plants offer an economic supplement.

David Spurgeon

correspondence

Spanish science: underfinanced and over centralised

SIR, — The uncertainty which research is suffering in Spain was briefly reviewed in a recent issue of *Nature* (9 August, p438). In the meantime we are approaching collapse.

In the last decade most research groups were supported by three-or four-year grants administered by the Comisión Asesora de Investigación Cientifica y Técnica. These grants are essential to cover running expenses as well as contracts for young scientists waiting for a stable position. The last distribution of grants took place in 1977 and all grants presently in existence terminate in December 1979. No new distribution scheme has been publicised, so that up to this moment we do not know the 1980 situation.

Even if a distribution of grants is announced in the next few weeks, processing them will take several months. Under these circumstances many scientists are forced to leave the country. Only emergency action will stop irreversible damage to research in Spain: namely, to extend automatically current grants for one year more.

The Ministy of Universities and Research is certainly preparing a three year plan (1980-1982) for the development of research in Spain. We are all eagerly waiting for its guidelines, but time is running short.

Another stumbling block in the development of research in Spain is its continuing centralism. A clear example is the Spanish Research Council (CSIC). Over two thirds of its activities and scientists are located in Madrid, with even a larger fraction of its budget concentrated in Madrid. This fact prevents a rational development of research elsewhere in Spain, close to places (industry, agriculture) where it is most needed.

In 1977, 187 new positions for scientists were provided; 70.6% of them were awarded to Institutes in Madrid. Presently another 198 new permanent positions are in the process of being provided; again over 60% of them will be for Institutes in Madrid. These numbers are not trivial, since the new positions mentioned represent 28% of the total permanent positions of the Spanish Research Council.

A peculiar geography of science has arisen through the years: there is not a single research institute in the Basque country, there are more Catalan biochemists working for the CSIC in Madrid than in Barcelona, there are more Catalan physicists working for the French CNRS than for the Spanish CSIC . . . This trend needs urgent modification. Drastic preference for new positions should be given to new and existing groups located in other areas of Spain.

At present a lack of funds and guidelines, together with the maintenance of centralism are producing irreparable damage to the development of research in Spain. We very much hope that the announced three year plan may open a brighter future.

Yours faithfully,

JUAN A SUBIRANA

Escuela Técnica Superior
de Ingenieros Industriales, Barcelona

Egyptian science

SIR, — I must comment on the article by Ziauddin Sardar (2 August, page 350).

"Divided and uncertain" does not reflect the reality of the Egyptian scientific community. As a scientist, and science editor for *Al Ahram* for the last twenty years, I will try to be objective, realistic and frank. The Egyptian scientific community, the biggest in the Arab world and Africa by any measure, is not divided regarding scientific cooperation with Israel. Instead the majority are for international scientific cooperation which they are practising all over the world in spite of shortcomings, drawbacks and loopholes in Egypt; and regardless of the sufferings of scientists and lack of stability and funds due to continous wars which have drained and exhausted our already depleted resources. Cooperation with Israel, on what basis?

In the meantime the powerful Israeli scientific community is silent and negative regarding the dangers threatening the peace process, mainly the insistence of the Israeli politicians and policy makers on depriving the Palestinians — including the scientific community — from their legitimate human rights to live in peace in their homeland. The Israeli scientific community is meanwhile raising hell against the Soviets for injustice against some individual Jewish scientists.

To be frank, the Egyptian scientific community does not like or agree with the Israeli superiority complex, the assumption that Israel is the only cradle of science and technology in the Middle East, and that it is an oasis of development and civilisation in a desert of backwardness inhabited by ignorant Arabs, including the Egyptians. Not bearing in mind the colossal funds and previleges Israel is getting from the US and other rich Jewish communities, the Israeli scientific community must realise and recognise that Egyptian scientists — inside Egypt and the brain-drained Egyptian scholars in the US, Europe and other Arab countries — are competent despite all the shortcomings. Unless confidence prevails, signed treaties will not help either Israeli or Egyptian scientific communities. We in Egypt refuse the Israeli arrogance in science and technology, including the assumption that Israel will teach the Egyptians and lead them to prosperity. Egyptians are certain (and not uncertain as Mr Sardar says) that comprehensive and just peace must include the Palestinians who have one of the highest rates of education of scientific personnel in the area, and we need their cooperation too, to build peace.

The Egyptian scientific community is certain and undivided about one thing: Egypt will still be part of the Arab world and cannot substitute Israel for Arabs, not only for religious, moral, ethical and historical reasons, but for practical, economic and psychological reasons too. The Arab world is the area where the Egyptian scientific community is practising and will continue to practise its human superiority, and make money too. This cannot be fulfilled in the cooperation with Israel.

Regarding Nechemia Meyer's article "Israel: a one sided love affair", I quite agree with the title but not with the content. The negative response to the willingness of the Israeli scientific community to enter into bilateral relations with Egyptian insitutions and the story of the experience of the Israeli scientists in Egypt is very normal. As long as the Israeli politicians and policy makers still insist on flagrantly depriving the Palestinians of their right to live peacefully in their homeland, and the negative attitude of the powerful Israeli scientific community continues, the chance of Egyptian-Israel scientific cooperation will be very marginal and meagre. Cooperation cannot be enforced by treaties, especially between scientists. Cooperation can start only when confidence is prevailing — and this is not the case yet. The Egyptian scientific community is part of Egypt, with all its aspirations for a just and comprehensive peace for Egyptians, Israelis and Palestinians.

Yours faithfully,

SALAH GALAL

Cairo

Highlighting persecution

SIR, — Dr Lorch (13 September, page 98) is right to emphasise the large themes of international solidarity. Vera Rich has from time to time highlighted smaller, but not unimportant, instances of persecution of individual scientists. Her facts are sometimes incomplete, but aren't they hard to come by? If Dr Lorch finds it easy, perhaps he would find out why the Fields Medallist Gregory Margoulis was not permitted to attend the Helsinki Congress of Mathematicians. Some of your readers would like to know.

As to the USSR relying on its own scientists, Dr Lorch will find an interesting example of masochistic circumcision if he looks at the USSR reproduction of *J. Chem.Soc.Faraday Transactions* II (69) 1973 page 1104; it deals with the . . . Dogonadze theory. The missing name is Levich.

Yours faithfully,

H. KESTELMAN

University College, London

Decentralising science

SIR,—This area is exactly such as you have in mind in your editorial 'Another sort of brain drain' (13 September). Apart from the excellent Agricultural Department at Aberystwyth 60 miles away, scientific and technological activity here is confined to teaching and the handful of people required to run the three oil refineries and the power station in Milford Haven.

Yet these massive capital intensive plants frequently find their way into lightly populated rural areas of the country, and could serve as a focus for a far more developed scientific community — enjoying incidentally lower establishment cost and infinitely preferable environmental regimes. It may not be fashionable or desirable to adopt the scientific cities of Soviet Siberia: but everyone would benefit from a sensible decentralisation of scientific work — not least from the elimination of time loss and stress inherent in daily travel to virtually any location in South East England.

Yours faithfully,

DAVID GREEN

Haverfordwest, Dyfed, UK.

news and views

Patterns in the abundance of parasites on plants

from Robert M. May

What determines the total number of parasitic species to which a particular species of genus of plants is host? This question—with 'parasite' interpreted broadly to range from herbivorous insects to fungi—has lately attracted much attention.

A primary factor is the geographical range of the host plant, with greater range implying more parasite species. As reviewed by Southwood (*Colloq. Int. du C.N.R.S.* **265,** Comportement des Insectes et Milieu Trophique, 471; 1977) and Strong (*A Rev. Entomol.* **24,** 89; 1979), various studies have shown that the logarithm of host-species range accounts for some 20-60% of the variation in the number of host-species for a panoply of associations including insects on trees in Hawaii, Britain and Europe, leaf miners of oak in California, gall-forming wasps on oaks in North America, arthropod pests of tea, cacao, and sugarcane, diseases of crops in the continental United States, and parastic fungi of British trees and North American plants. Three recent papers go beyond this, seeking to understand the causes of the residual variation in 'species richness' of parasites, after such 'species-area' effects are factored out: Strong and Levin (*Amer. Nat.* **114,** 1; 1979) analyse an eclectically compiled array of data on the insect associations of 214 genera of British plants and on the fungal parasites of 323 species of US plants; Lawton and Schroder (*Nature* **265,** 137; 1977) examine the insect fauna of some 70 species of British herbs and shrubs; and Lawton and Price (*J. Anim. Ecol.* **48,** 619; 1979) deal with the narrower group of larval agromyzids that mine the leaves and stems of some 60 plant species in the family Umbelliferae in Britain.

Strong and Levin divide the plants into trees, shrubs and herbs. They show that there is no significant difference among these three categories in the way species richness of parasites increases with geographical range of the host plant, but that trees systematically tend to have more parasitic species than do shrubs, which in turn have more than herbs (per unit area of geographical distribution). More precisely, their 31 genera of British trees have an average of 28 species of insects, 40 genera of shrubs average 10 insect species, and 143 genera of herbs average around 5 insect species. The number of species of parasitic fungi average around 15 for their 127 species of North American trees, 7 for 70 shrub species, and 5 for 126 herb species. Lawton and Price classify their umbellifers according to the mean physical size of the plant (the geometric mean of the normal maximum and minimum heights of mature plants, as given in Clapham, Tutin and Warburg's *Flora of the British Isles)* and according to leaf form; these are both measures of the 'architectural complexity' of the plant. They will find that species-area effects account for 32% of the variance in species richness of mining insects, and that plant architecture explains some 20% of the remaining variance.

Within any one of their three growth-form categories, Strong and Levin find that most further comparisons reveal no discernible differences in species richness of parasites. Thus American conifers and flowering trees support roughly the same number of species of parasitic fungi, and grasses do not differ significantly from herbs in general. Likewise, British monocots, grasses, aquatic and bog plants, sedges and composites do not differ discernibly from herbs in general in the number of insects species they support per unit host range. Classifying the host plants by life-history strategy, Lawton and Price were unable to determine any significant difference in the number of agromyzid species associated with annual, biennial or perennial umbellifers; in this repect the umbellifers resemble European Cynarae (Lawton and Schroder, *Proc. IV Int. Symp. Biol. Control of Weeds,* ed. T.H. Freeman, University of Florida Press, 1978). Two comparisons within the herb-shrub-tree categories do, however, show differences; ferns support fewer insect species than do other herb genera in Britain; and weedy herbs support fewer fungal parasites than do climax and subclimax herbs in the US. I shall return to these two points below.

Strong and Levin summarise their results by proposing "that the greater size and morphological complexity of trees than shrubs, and than herbs in turn, account for these patterns in pest species richness; larger more complex hosts present a greater diversity of pest niches". This matches with Lawton and Schroder and with Lawton and Price's suggestion that plant 'architecture' is a significant component in accounting for patterns in the number of parasitic species, a theme which is further explored in several of the papers in the Special Symposium on Insect Diversity held in 1977 by the Royal Entomological Society (see the chapters by Southward, Lawton and May in *Diversity of Insect Faunas,* eds Mound & Waloff, Blackwell, 1979).

The finding that ferns support fewer species of parasites than do other herbs can be challenged on the grounds that the fauna of ferns are typically less well studied than those of seed plants. But a meticulous study of the invertebrate fauna of the fern *Pteridium aquilinum,* or bracken, in Britain (Lawton *Bot. J. Linn. Soc* **73,** 187; 1976) suggests that ferns do indeed possess a relatively impoverished fauna of insect pests; adjusted for its range in Britain, bracken does not have a rich pest fauna relative to other herbs, even with Lawton's extremely complete data. Lawton's study encompasses the chemistry of bracken, suggesting it has five distinct modes of chemical protection. It may be that ferns generally possess a greater diversity of biochemical defences than do other herbs, which affords them relative freedom from pests.

The conclusion by Strong and Levin that weedy herbs tend to have fewer fungal parasites than other US herbs is supported by Lawton and Schroder's finding that weeds and other annuals tend to have fewer insect pests than perennial herbs. This tendency is, however, not manifested among Lawton and Price's umbellifers. Moreover, the data for fungal species on weedy herbs is very noisy, in the sense that the species-area regression accounts for only 12% of the variance (in contrast to 30-50% for other herbs, shrubs and trees). The question is further clouded by the fact that the man-made disturbances, which have greatly increased habitat for weeds and spread them so widely throughout the US, have mainly occurred only over the

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Robert M. May is Class of 1877 Professor of Zoology at Princeton University.

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past 200 years. All this is a pity, as patterns in the relative number of parasitic species associated with weedy herbs could shed light on Feeny's *(Rec. Adv. Phytochem.* **10**, 1; 1976) ideas about plant 'apparency'. According to this hypothesis, 'apparent' plant species (which are large, more long-lived, and tend to occur later in succession) are "bound to be found" by herbivores; they therefore need general (quantitative) defences that reduce digestion in herbivores and that cannot easily be breached by the ability of parasites to evolve resistance to plant-produced poisons. Conversely, ephemeral or 'inapparent' plants emphasise reproduction and dispersal, and minimise the metabolic cost of defence against herbivores by use of (qualitative) poisons that are effective in small doses. It is not clear what these ideas imply for the correlation between species richness of parasites and host plant 'apparency'; as Strong and Levin observe, "The null hypothesis that chemical differences do not result in different numbers of parasite species for plant taxa should be tested. One way of doing this might be to look for patterns within residuals about the pest species/host area regression line for each growth form". More work is needed before any generalisation can be made here.

In short, something like 50% in the variance of species richness of parasites among host plants can typically be attributed to the geographical range and architectural complexity of the host. Strong and Levin, and Lawton and Price, advance various suggestions about the other 50%. Lawton and Price favour the idea "that the subtle accumulation of specialists (e.g.miners and gall formers) may continue over very long periods of evolutionary time". This could result in much variability if their 60-odd agromyzid-umbellifer associations were at many different stages along the road to eventual equilibrium. Most of the evidence, however, suggests that the total number of parasite species on host taxa usually reaches an asymptote within short periods of evolutionary time (see for example, Gilbert in *Analysis of Ecological Systems*, eds Horn, Mitchell & Stairs, Ohio University Press, 1979); Strong, *op. cit.* 1979). Other explanations for the residual variability include incomplete knowledge of the parasite fauna, differences in the abundances of plant species of equal geographical range (common plants could well have more species of parasites than rare ones, geographical ranges being equal), and variation in the efficiency of the plant's biochemical defences. On top of all this is the possibility, recently and cogently emphasised by Simberloff and others, that substantial differences arise from historical and evolutionary accidents, and have no discernible deterministic cause.

Stepping away from the details, we can see these thoughtful and technically sophisticated studies as a paradigm of much of the contemporary search for patterns in ecosystems. Depending on one's cast of mind, one can derive pleasure from the systematic explanation of roughly half the variability in the number of parasite species on plants, or pain from that half of the variability that remains unexplained. □

Dissecting the red cell membrane skeleton

from Samuel E. Lux

To survive in the circulation red blood cells must be both durable and flexible: durable enough to withstand the turbulent cardiac passage and flexible enough to negotiate the narrow portals of the spleen. These dual demands are the responsibility of a submembranous protein network which functions as a 'membrane skeleton'. Because defects in this structure are probably responsible for a number of important haemolytic anaemias (see Lux *Semin. Hematol.* **16**, 21; 1979 for a review) and because it is a model for cytoskeletons of more complex and less accessible cells, a great deal of effort has been expended dissecting and reassembling the skeleton of the human red cell. This analysis is currently proceeding at an exceptional pace and seems, at last, to be leading to a comprehensible picture of skeletal anatomy.

Definition of the membrane skeleton

Operationally, the membrane skeleton is defined as the insoluble residue which remains after extraction of intact red cells or their isolated membranes with the nonionic detergent Triton X-100. Under the electron microscope this structure is an anastomosing network of twisted, irregularly oriented microfilaments dotted with globular protrusions (Hainfeld & Steck *J. supramolec. Struct.* **6**, 301; 1977; Liu *et al. Fed. Proc.* **7**, 1508; 1978). It comprises about 60% of the membrane protein mass and includes all of the spectrin (bands 1 and 2), actin (band 5), ankyrin (bands 2.1, 2.2, 2.3 and 2.6) and band 4.1; and a portion of the proteins designated bands 3, 4.2, 4.9 and 7 (Fig. 1). Spectrin, actin and bands 4.1 and 4.9 form the core of the structure since the skeleton retains its shape and structure when the other components are eluted with hypertonic KCl (Sheetz *Biochim. biophys. Acta,* in the press) but disintegrates if either spectrin or actin are removed (Sheetz *J. Cell Biol.* **81**, 266; 1979).

Spectrin structure

Spectrin, the major skeletal protein (60 to 75% by weight), is composed of two enormous subunits, bands 1(molecular weight 240,000) and 2 (220,000) which are structurally similar but are not identical (Anderson *J. biol. Chem.* **254**, 939; 1979). The protein has been intensively analysed since its discovery by Marchesi and Steers more than a decade ago (*Science* **159**, 203; 1968). In the past few years purified spectrin heterodimers (band 1+2) and heterotetramers (band $1+2)_2$ have been isolated and sized (Ralston *Biochim. biophys. Acta* **455**, 163; 1976; Kam *et al. Biochemistry* **16**, 5568; 1977), the equilibrium relationships of these molecules have been defined (Ungewickell & Gratzer *Eur. J. Biochem.* **88**, 379; 1978), and the tetramer has been identified as the physiological species. However, because spectrin has unusual hydrodynamic properties and is difficult to see in the electron microscope, two of the most critical pieces of information — the shape and molecular conformation of the molecule — have resisted definition. Fortunately, Shotton, Branton and their colleagues (*J. molec. Biol.* **131**, 303; 1979) have recently discovered that spectrin's molecular outlines can be captured in platinum-carbon replicas formed by low-angle shadowing. These molecular casts clearly show that spectrin dimers are long (~1000 Å), slender (~ 50 Å), worm-like molecules in which the two subunits are aligned in parallel and variably coiled about each other. Individual molecules assume many different conformations and must be quite flexible — a property which may be critical for normal membrane pliancy. Spectrin dimers join end to end to form tetramers, but high order oligomers are not seen. This confirms the work of Ralston and Kam noted above and indicates that the dimers join head to head instead of head to tail, since, in the latter alignment at least some hexamers, octamers, and so on, should be formed. Because spectrin subunits seem to be tightly attached at their extremities, Shotton postulates that complementary sites exist at the ends of each of the sister subunits and suggests that the dimer-tetramer interconversion simply involves a reorientation of the sites at the heads of two neighbouring dimers so that each subunit pairs with its sister chain on

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Samuel E. Lux is Associate Professor of Pediatrics at Harvard Medical School and Division of Hematology-Oncology, Children's Hospital Medical Centre and Sidney Farber Cancer Institute, Boston.

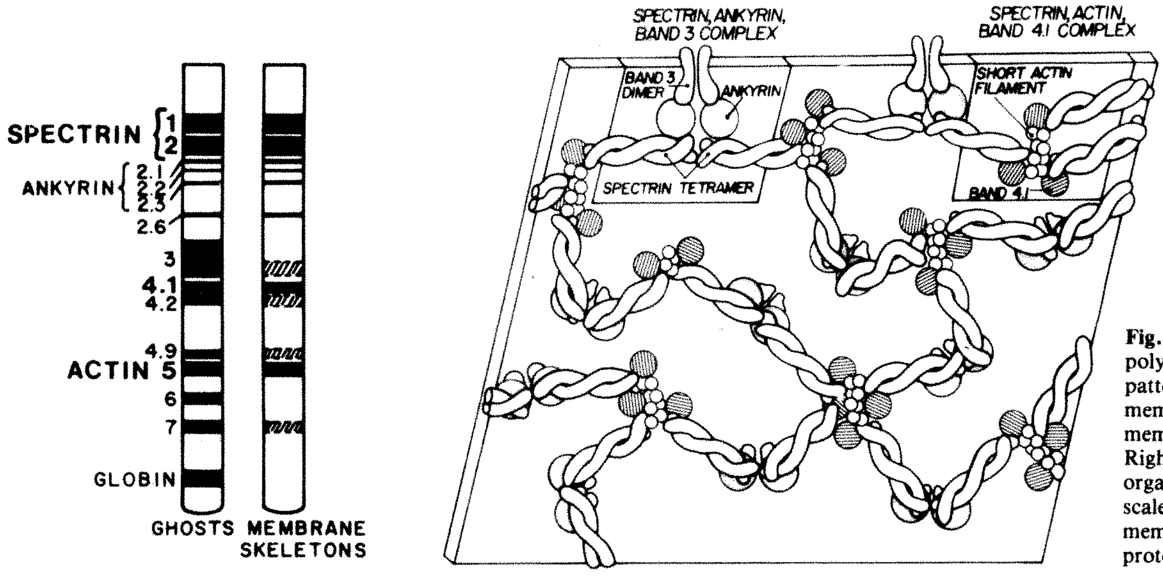

Fig.1 Left, SDS polyacrylamide gel patterns of red cell membranes (ghosts) and membrane skeletons. Right Postulated organisation (not to scale) of the major membrane skeletal proteins.

the adjacent dimer. This is an attractive hypothesis since the cleavage of existing bonds (the first step in the dimer–tetramer interconversion) could account for the unusually high activation energy of the process (Ungewickell & Gratzer *op. cit.*).

The spectrin-actin-band 4.1 complex

If pure spectrin dimers can only associate to tetramers, then clearly other molecules are required to form the basic skeletal framework. Their identification has been approached in two ways: by dissection and reassembly. Sheetz finds he can strip away all of the skeletal proteins except spectrin, actin and bands 4.1 and 4.9 and still retain an intact skeletal substructure. This suggests that one or more of the latter three proteins provides the critical cross-linking function. Studies reported recently in *Nature* (**280**, 811; 1979) confirm this postulate. Ungewickell and his colleagues observe that mixtures of purified spectrin tetramer, polymerised actin (F-actin) and band 4.1 combine at physiological ionic strengths into pelletable ternary complexes and, in some cases, gel. No complexes are formed from binary mixtures of these proteins or from ternary mixtures if the actin is unpolymerised (G-actin). Electron micrographs of isolated complexes show F-actin filaments bridged by fine curved fibrils which presumably represent spectrin tetramers. The pictures suggest the actin-binding regions of the tetramer are near the distal (tail) ends of the molecule. Dimeric spectrin attaches to actin filaments but, being monofunctional, is unable to cross-link them.

Independent evidence from several other laboratories largely corroborates this picture. Fowler and Taylor (*J. Cell Biol.* **79**, 222a; 1978) have reported that oligomeric complexes of spectrin, actin and band 4.1, extracted from red cell membranes cause actin to gel and that purified spectrin dimers or tetramers lack this activity, suggesting a requirement for band 4.1. More recently (personal communication) they discovered that this effect can be mimicked simply by adding purified tetramer and band 4.1 to actin solutions. Interestingly, they find the reaction is maximal at physiological concentrations of calcium (10^{-7}–10^{-8} M), which may explain, at least in part, the deleterious effects of this ion when it accumulates in red cells. At lower ionic strengths, Cohen and his coworkers (personal communication) observe that purified spectrin induces actin gelation in the absence of detectable band 4.1 and Tyler and Branton (*Proc. natn. Acad. Sci. U.S.A.*, in the press) report that purified band 4.1 binds to spectrin in the absence of actin, indicating that stable binary complexes can exist under certain circumstances. Electron micrographs of spectrin–4.1 complexes show that the binding site of band 4.1, like that of actin, is located near the tail of the spectrin dimer.

These studies are all consistent with a skeleton composed of long, flexible, bifunctional, spectrin tetramer strands cross-linked at each end through interactions with actin and band 4.1 (Fig. 1). While this model of the skeleton is greatly advanced compared to earlier renditions, many important questions remain. For example, what is the state of the actin? The experiments of Ungewickell *et al.* and observations of Brenner and Korn (*J. biol. Chem.* **254**, 8620; 1979) indicate that actin must be polymerised to complex with spectrin. But actin filaments are not visible in electron micrographs of red cell membranes and the amount of actin present (about four molecules per spectrin tetramer) is not compatible with the existence of long actin filaments. This leads to the conclusion that actin must exist in short filaments or 'protofilaments' (Cohen & Branton *Nature* **279**, 163; 1979; Ungewickell *et al. op. cit.*), and, by extension, implies that some mechanism must exist to limit the extension of such filaments. The point is by no means settled however, since there is evidence that oligomeric complexes of spectrin, actin and band 4.1 may contain some G-actin (Ralston *Biochim. biophys. Acta* **510**, 283; 1978). Nevertheless, at present it seems likely that much of the actin in the skeleton is organised as short filaments. If true this leads to other questions. Are all these filaments the same size? Are the number of spectrin tetramers which branch from the filaments constant or variable? In the model shown in Fig. 1, I assume that variable numbers of spectrin molecules are joined by actin filaments of variable length. This hypothesis would explain the disordered organisation of the skeleton seen by electron microscopy, but other explanations are also possible. For example, the disorder could simply be due to the inherent flexibility of the spectrin molecules. Alternatively spectrin may have multiple binding sites for actin and/or band 4.1 near its distal end which are variably filled.

The spectrin-ankyrin-band 3 complex

The interaction of the red cell skeleton with the overlying lipid bilayer is better understood than the organisation of the skeleton itself, thanks largely to a series of elegant studies which originated in the laboratory of Daniel Branton at Harvard. The story began with the observation that the red cell contains about 100,000, high affinity (K_D = 10^{-7} to 10^{-8} M) binding sites for spectrin on its inner surface (Bennett & Branton *J. biol. Chem.* **252**, 2753; 1977). Subsequent chapters revealed that each of the sites resides in a 72,000 molecular weight chymotryptic fragment (Bennet *J. biol. Chem.* **253**, 2292; 1978), that antibodies to this fragment label only bands 2.1 and 2.2 (Bennet & Stenbuck *J. biol. Chem.* **254**, 2533; 1979), and that peptide maps of these two bands and bands 2.3 and 2.6 are homologous with the 72,000 molecular weight piece (Luna *et al. J. biol. Chem.* **254**, 2526; 1979; Yu & Goodman *Proc. natn. Acad. Sci. U.S.A.* **76**, 2340; 1979). This suggests that the lower molecular weight piece (Luna *et al. J. biol.* products of the parent band 2.1 molecule. At present it is uncertain whether they are formed in intact red cells or are an artefact of membrane preparation.

Band 2.1 and its progeny have been variously named ankyrin (Greek, anchor), nectin (Latin, to connect), and syndein (Greek, to bink together). Ankyrin was chosen first, but because this name had already been given to a plant protein and because it soon became clear that ankyrin was not the true anchor (see below), the protein was rechristened nectin. Unfortunately this name was also a duplicate, so a second change to syndein was made. Some of those working in the field continue to use ankyrin while others have adopted the revised terminology. Presumably the rest of us will have to choose between these appellations (I prefer ankyrin) or make up our own, at least until some Commission on Nomenclature decrees.

In the latest sequel to this saga (*Nature* **280**, 468; 1979), Bennett and Stenbuck report that ankyrin is firmly linked to band 3 in detergent extracts of spectrin-depleted red cell membranes. (It should be noted that some band 4.2 is also associated with band 3 in these extracts. The data presented favour the author's interpretation of a direct linkage between ankyrin and band 3, but do not completely exclude the possibility that band 4.2 is interposed between these two proteins). The band 3 molecules involved (10–20% of the total) are apparently identical to the principal band 3 protein which functions as an anion exchange channel. Unlike ankyrin this protein spans the lipid bilayer and is presumably the true site of skeletal anchorage. Ankyrin is thus not an anchor but an anchor chain — a protein that links spectrin and band 3. In fact it may function generally to connect cytoskeletal elements and integral membrane poteins since, unlike spectrin (see below), it is apparently present in many types of cells (Bennet & Stenbuck *op. cit.*).

The electron microscopic techniques used to visualise the band 4.1 binding site have also been applied to ankyrin (Tyler & Branton *op. cit.*) and are even more successful since ankyrin is a larger molecule (a 80 Å × 95 Å pyramid) than band 4.1 (a 60 Å sphere). These studies clearly show that ankyrin binds to a site 200 Å distal to the end of spectrin involved in the dimer-tetramer interaction. The stoichiometry of this linkage (whether there are one or two ankyrins per tetramer) remains to be determined.

Membrane skeletal model

Taken together, this information provides a fairly clear picture of how the principal skeletal proteins are probably organised (Fig. 1). Certain general points remain to be established (for example, the stoichiometry of the spectrin-actin-band 4.1 and spectrin-ankyrin-band 3 complexes and the physical state of actin), and many structural details must still be pursued, but the general framework appears to be taking shape. It must be emphasised however, that the true skeleton is undoubtedly more complicated than the model shown in Fig 1. Skeletal proteins like bands 4.2, 4.9 and 7 are not included in the model since too little is known about their location and function. Band 4.1 must attach to the bilayer in some way since most of it remains on the membrane when spectrin and actin are extracted at low ionic strength. And spectrin chains must often cross each other since the average spacing between ankyrin-linked band 3 dimers (~375-530 Å) is considerably less than the average length of a spectrin tetramer (~2000 Å).

Phosphorylation

Most of the membrane skeletal proteins

Halley's Comet in ancient history

from David W. Hughes

RECENT books on comets state categorically that the first definitely recorded return of Halley's comet was that of 240 BC, some adding as a postscript that there is a vague possibility that comets described in 613 BC and 467 BC may also have been P/Halley (the P/prefix indicating a periodic comet). It now seems, however, that observations go back considerably further. Y. C. Chang of the Purple Mountain Observatory, Nanking, China finds that the earliest astronomical record of the comet in the historical annals of his country described the apparition of 1057 BC. Reasons for this assertion, together with other early Chinese observations, are published in Chang's recent paper in *Chinese Astronomy* (**3**, 120; 1979) (a translation of *Acta Astr. Sinica* **19**, 109; 1978).

The problem of recognising the historical record as a description of Halley's comet has been approached in two ways. The first relies on celestial mechanics to produce an accurate analysis of the orbital evolution of the comet. Chang started with the position and velocity vectors of Halley's comet and the eight major Solar System planets for a specified epoch (Julian day 2430000.5). The cometary results were taken from Brady and Carpenter (*Astr. J* **76**, 728; 1971) who based their findings on 3085 observations of P/Halley made by 60 observatories over an unbroken period of 20 months during the 1910 return.

The equation of motion of the comet around the Sun was then integrated, taking into account gravitational perturbations by the planets. This enabled the perihelion passage times and orbital elements of P/Halley to be established for the previous 3000 years. So Chang knew when and where in the sky the comet would have appeared. The planetary perturbations produce changes in the orbital period of the comet, which was for example about 72.9 years between 762 BC and 689 BC, and as long as 79.7 years between AD 1222 and AD 1301. The inclination of the orbit has decreased by about 1.5° over 3000 years, and during this time the argument of perihelion has changed from 77° to 112° and the longitude of the descending node from 18° to 58°. A more important parameter is the maximum brightness of the comet. This seems to change by about 2.7 magnitudes per millennium, indicating that P/Halley will reach a maximum magnitude about +0.5 at its next apparition in 1986, whereas it was about −7.7 in 1057 BC — brighter than any of the planets.

Brady (*Pub. Astr. Soc. Pacific* **83**, 314; 1972) also studied the orbital evolution of P/Halley and suggests that the present planets could not account for all the observed changes. To overcome this difficulty he predicted the existence of another planet at a distance of 64 astronomical units (AU) from the Sun, with a mass three times that of Saturn. Chang does not confirm this hypothesis and finds that the known planets account for the observed perturbations. Some astronomers have also proposed that there is a cloud of comets about 50 AU from the Sun having a total mass similar to that of the Earth. Even though P/Halley, with its aphelion distance of 35 AU, would spend a considerable time near this cloud, Chang again finds that such a cloud is unnecessary when it comes to accounting for the perturbation of P/Halley.

Chang's second approach was to peruse the Chinese astronomical records, the most complete historical records in existence, for accounts of Halley's comet. The new account came to light in a chapter on military strategy in the *Book of Prince Huai Nan*. This recounts how a comet was seen in the east with its tail pointing to the northwest at the time when King Wu marched on Zhou of Yin. The book also gives the position of Jupiter at that time. There are three schools of historical thought about the time of Wu's campaign, favouring 1122 BC, 1075-1055 BC or 1027 BC. All agree however, that it occurred in the first two months after the winter solstice. By combining these factors Chang concluded that the campaign and the observation of Halley's comet took place in the winter of 1058/57 BC, so our next observations of P/Halley in 1985/86 will not be the thirtieth, as previously thought, but the forty-first recorded apparition.

David W. Hughes is a Lecturer in the Department of Physics, University of Sheffield.

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are phosphorylated in the intact red cell (Plut *et al. Eur. J. Biochem.* **82,** 333; 1978). Both cyclic AMP-dependent (ankyrin, bands 4.1, 4.9 and 7) and cyclic AMP-independent (spectrin, band 3) kinases are involved. In spectrin, the best studied example, four sites are labelled in a cluster near one end of the band 2 subunit (Harris *et al. Fed Proc.* **37,** 1507; 1978; Anderson *op. cit.*). This is probably the end of the molecule where actin and band 4.1 bind since interactions between actin and crude spectrin preparations are phosphorylation dependence in more purified systems (Brenner & Korn *op. cit.*; Cohen & Branton *op. cit.*). Nevertheless, Pinder's results strongly suggest that phosphorylation does modulate spectrin-actin-4.1 interactions in some way. Presumably it is also functionally important to the other phosphorylated skeletal proteins. Deciphering these functions and how phosphorylation affects them is probably the major unsolved issue in the field.

Evolution

Spectrin (and by implication a spectrin network) is present in all mammalian and avian red cells but has not been detected in other cell types. Pinder and her associates have even found a spectrin-like molecule, immunochemically homologous to human spectrin, in red cells from primitive invertebrates *(FEBS Lett.* **92,** 278; 1978). This implies that spectrin has evolutionarily critical functions and, since its only known functions relate to the membranes vesiculate frenetically when evolutionarily critical structure. This is understandable from the chauvinistic perspective of the spectrinologist; red cell membranes vesiculate freneticlly when spectrin is genetically absent (Greenquist *et al. Blood* **51,** 1149; 1978) or purposely removed and presumably have required support from their inception. What is not clear is how other cells survive without it! Perhaps analogous proteins have evolved to stabilise their membranes but, if so, they remain to be identified. Alternatively nonerythroid cells may have found their ubiquitous cytoskeletal scaffolding sufficient for membrane support while red cells, anxious to discard their intracellular organelles to accommodate haemoglobin, were forced to invent spectrin. Whatever the reason, it remains a mystery (at least to me). □

Genetic approaches to neurobiology

from Gilbert S. Omenn & John P. Blass

THE concepts and techniques of genetics are being wedded to those of the neurosciences with increasing success. Studies of genetically defined lesions in invertebrates, mice, humans, and cultured cells are providing descriptive and pathogenetic information about complex brain functions and behaviours. Several productive new lines of research at this interface were reported at an International Society for Neurochemistry (ISN) meeting in Greece*.

Deliberate construction of mosaic or mutant mice now offers a powerful way to investigate models of human disorders and to distinguish whether dysfunction is intrinsic or extrinsic to the nervous system or muscle. B. Mintz (Institute for Cancer Research, Philadelphia), who pioneered the use of allophenic mice (formed by agregating cells from two 8-cell embryos from two different pregnant mice, hence tetraparental), has constructed such mice using mouse teratocarcinoma (TC) cells in place of one pair of parents. The TC cells serve as a remarkable vehicle for introduction of specific marker or mutant genes, obtained by any of the techniques of somatic cell genetics including mutagen treatment and selection, recombinant DNA techniques, and cell fusion. Mintz has created an $\text{HGPRT}^-/\text{HGPRT}^+$ mosaic mouse from a normal embryo plus HGPRT^-TC cells. Completely HGPRT^- progeny generated from these mice will be the first true animal model for the fascinating and perplexing human inborn error of metabolism, Lesch-Nyhan disease. In Lesch-Nyhan disease, deficiency of HGPRT (hypoxanthine guanine phosphoribosyltransferase) as a result of a single structural gene mutation leads to complex behavioural pathology, with compulsive aggressiveness, and neurological abnormalities. HGPRT alleles with varying residual enzyme activity could be used to generate a graded series of HGPRT deficiencies for neurochemical and behavioural correlations and it is likely that a whole array of specific mutant mice will be constructed in the years ahead.

A. Peterson (McGill University, Montreal) described allophenic mosaic mice (strains C57BL and 129) constructed to test whether muscular dystrophy (*dy/dy*) in the mouse is of primary muscle or primary neurogenic origin. Mosaics between strains 129 and C57BL were observed to have muscle entirely of 129 origin (according to marker enzymes), while the rest of the animal is mosaic. Thus, Peterson could construct mosaic mice with *dy/dy* muscle; such genotypically abnormal muscle proved to be entirely normal on examination, indicating an extramuscular mechanism. If the complementary mice with genotypically normal muscle in a *dy/dy* C57BL × +/+ 129 mosaic develop the typical dystrophy, non-myogenic origins of the disorder will be proved. This genetic innovation may resolve a long-standing controversy in neurobiology.

Animal mutants continue to provide valuable insights for developmental neurobiology. A dysmyelinating mutant, for example, the *shiverer* mouse, lacks immunochemically detectable myelin basic protein, according to N. Baumann (Salpetrière, Paris) and A. Tsukada (Keio University, Tokyo). The compaction of lamellae is abnormal, but myelin is assembled despite the absence of basic protein, and the animal survives. A whole series of dysmyelinating mutants affecting central and/or peripheral myelination is now under comparative neurochemical investigation.

Invertebrates, especially nematodes (*C. elegans*) and *Drosophila*, are well-suited for genetic studies of certain limited kinds of behaviour. However, despite extensive anatomical and genetic analyses, neurochemical findings are negligible in nematodes and are only just emerging in *Drosophila*. Y. Dudai (Weizmann Institute, Rehovoth, Israel) described current studies of the *Drosophila* learning mutant '*dunce*', which appears to lack one isoenzyme of cyclic AMP phosphodiesterase. The gene for this enzyme maps on the X chromosome to exactly the same position as the *dunce* mutation. Knowledge of the chromosome map and availability of deletion mutants made possible this correlation. Direct assays (D. Byars, CalTech) indicate raised cyclic AMP concentrations in these flies. Obviously, there is still a gap between finding a phosphodiesterase deficiency and explaining the defect in learning olfactory differences.

Human mutations have long fertilised neurochemistry, with Tay-Sachs disease as the classic example. Gangliosides were discovered in brain tissue from patients with Tay-Sachs disease (TSD), who accumulate large amounts. The sequential nature of normal degradation of sphingolipids was revealed by recognition and analysis of a whole series of human lipid storage diseases, each due to a single gene mutation and resultant enzyme deficiency.

K. Sandhoff (University of Bonn) demonstrated anew that studies of these mutations can advance basic biochemistry of the brain. He described an endogenous activator protein essential to the physiological action of hexosaminidase A (deficient in TSD) on lipid soluble substrates in micelles *in vitro*. Presumably, this activator is crucial for Hex A action on lipids within membranes *in vivo*. In clinical neurochemistry, the activator protein

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*Symposium on Genetic Approaches to Neurobiology and Neurological Disorders, University of Patras School of Medicine, Patras, Greece, 30-31 August, 1979; official satellite Symposium of Congress of International Society for Neurochemistry, Jerusalem, 2-6 September, 1979. Organisers were S. Doxiadis, P. Kafatos, E. Kouvelas, A. Simopoulos, J. P. Blass and G. S. Omenn.

G. S. Omenn is Associate Director, Office of Science and Technology Policy, Washington, DC and J. P. Blass is Professor of Neurology, Cornell University, New York.

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effect can explain the previously perplexing patterns of accumulation of gangliosides and globosides in patients with the various forms of TSD; Hex A, but not Hex B, is strikingly activated. Finally, three TSD patients with apparently normal Hex A activity (AB variant) were found to lack the Hex A-activator protein. It is very likely that analogous activator proteins specific for sulphatides and other lipids exist. They may prove to have interesting protein structures, and a sulphatide-specific activator protein may account for known cases of metachromatic leukodystrophy (MLD) with apparently normal activity of the arylsulphatases.

These highlights illustrate the power and sensitivity of genetic approaches to studies of complex functions of the nervous system and behaviour.

As this Symposium was an integral part of the Jerusalem meeting of the ISN, it is significant to note that Professor Ekram Abdel Salam of Cairo University and Professor Yadin Dudai of the Weizmann Institute were both active participants. In the near future, we expect that such contacts will be made directly in Egypt and in Israel. ☐

Interstellar molecules

from B. Zuckerman

AFTER a decade of explosive growth, the study of interstellar molecules has reached a certain level of maturity. That seemed to be the picture that emerged from a recent symposium at which the organisers rather successfully blended a combination of astronomers, physicists, chemists and spectroscopists. That they were perhaps not entirely successful was suggested during the presentation of one European astronomer, when a "thank you" from the speaker directed to the slide projectionist woke up a sizable fraction of the audience who then proceeded to deliver an unexpected round of applause.

The startling new results that characterised the early 1970s were absent from this symposium. Still there are many controversies and mysteries yet to be unravelled. In spite of the very low temperatures and fairly quiescent states that characterise most interstellar molecular clouds there is evidence of surprisingly energetic phenomena hidden deep inside some clouds. S. Beckwith (Cornell University), T. Geballe (California Institute of Technology), N. Scoville (University of Massachusetts, Amherst) and D. Hollenbach (NASA Ames Research Center) discussed high velocity gas and shock waves in the cloud behind the Orion Nebula. The very large energies associated with the high velocity gas imply the existence of massive stellar winds or an explosive event (perhaps a supernova) inside this molecular cloud.

Even for the more typical molecular cloud, J. Silk (University of California, Berkeley) and C. Norman (University of Leiden) suggested that support against gravitational collapse can best be provided by energetic winds from new stars similar to the Sun that have just formed inside the clouds. That molecular clouds are somehow stabilised against free-fall gravitational collapse now seems generally, although perhaps not universally, accepted. Indeed the present controversy rages around the question of whether the giant molecular cloud complexes 'live' for at least a few times 10^8 years (P. Solomon, State University of New York, Stony Brook) or 'only' a few times 10^7 years (L. Blitz, University of California, Berkeley). In the former case the molecular clouds are well distributed throughout the inner disk of our Milky Way Galaxy, in the latter case they are confined to the spiral arms. Future observations of carbon monoxide in external galaxies should resolve the matter.

A renaissance in this field awaits development of sensitive new receivers especially for wavelengths $\leqslant 1$ mm to be used with large new mm wavelength telescopes and interferometers presently under construction or envisaged. Previews of some of the exciting results to be expected at high frequencies were given by P. Vanden Bout (University of Texas) and by T. Phillips (Bell Telephone Laboratories). Phillips reported submillimetre observations of CO (at 461 GHz) and of H_2O (at 380 GHz) obtained from NASA's Kuiper Airborn Observatory.

One of the fundamental limits in this field is the total number of different interstellar molecules that may eventually be found by radioastronomers. P. Thaddeus (Goddard Institute for Space Studies, New York) pointed out that 52 interstellar molecules have now been identified but, from considerations of the limits set by confusion due to overlapping weak lines from various molecules, he estimated that probably only about three times this number would ever be identified. Even so, the microwave spectroscopists will have their work cut out for them if we are to assign the observed transitions properly. ☐

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*The Symposium on Interstellar Molecules was held at Mont Tremblant, Quebec on 6-10 August and sponsored by the International Astronomical Union in conjunction with the Herzberg Institute of Astrophysics and the National Research Council of Canada.

B. Zuckerman is Professor of Astronomy in the Astronomy Program, University of Maryland.

The end of the Cretaceous

from A. Hallam

IT is widely recognised that the end of the Cretaceous witnessed one of the most significant mass extinction events in Earth's history, both on land and at sea. Whereas certain groups like the ammonites had been in decline for some time, others such as the dinosaurs and planktonic foraminifers were still flourishing until the end of the period. The many hypotheses that have been proposed to account for this striking extinction event fall into two categories, respectively invoking extraterrestrial and terrestrial causes.

The extra-terrestrial or 'astronomical' hypotheses involve increased levels of some form of unpleasant radiation from outer space, perhaps at times of geomagnetic reversals. They have been strongly criticised and are not widely accepted today but the general notion cannot yet be decisively discounted and has been recently revived by Reid *et al.* (*Nature* **259**, 177; 1976; **275**, 489; 1978), who suggest increased ultraviolet radiation at the Earth's surface as a result of breakdown of the protective ozone belt, caused by increased solar proton flux or supernova explosions. One of the strongest arguments against such radiation events to account for various mass extinctions in the past is that terrestrial floras were comparatively unaffected. It is noteworthy therefore that Krassilov (*Palaeontology* **21**, 893; 1978) has recorded an abrupt change in terrestrial floras across the Cretaceous-Tertiary boundary.

The 'terrestrial' group of hypotheses usually postulate some form of sea level and/or climatic change. There is good stratigraphic evidence of a major eustatic regression at the end of the Cretaceous, and such regressions plausibly account for a number of mass extinction episodes of shelf faunas in the Phanerozoic, but it is not so evident why planktonic and terrestrial groups should be seriously affected. One ingenious solution to this problem was proposed some years ago by Tappan (*Palaeogeog., Palaeoclimatol., Palaeoecol.* **4**, 187; 1968). She postulated that the production of marine phytoplankton controlled the oxygen and carbon dioxide levels of the atmosphere through time, and that changes in relative and absolute abundance of these organisms had extensive effects on contemporary biota. Following the major late Cretaceous transgression which preceded the end Cretaceous regression, the influx of nutrient supply from land dropped as continents were reduced to base level. This and other associated processes led to a marked reduction in marine phytoplankton and a consequential

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A. Hallam is Lapworth Professor of Geology in the University of Birmingham.

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increase of atmospheric carbon dioxide and decrease of oxygen (due to reduced photosynthesis). These atmospheric changes were too much for the dinosaurs to cope with, though estivators and hibernators such as the crocodiles and turtles were able to survive into the Tertiary.

Data from new fields of research have now been brought to bear on the problem. Geomagnetic reversals as recorded in sedimentary sequences both on land and under the ocean can be used to add precision to stratigraphic correlation based on fossils. A biostratigraphically complete sequence of Upper Cretaceous and Palaeogene pelagic limestones at Gubbio, Italy has produced a record of geomagnetic reversals that closely matches the marine magnetic anomaly sequence (Alvarez *et al.*, *Bull. geol. Soc. Am.* **88**, 367; 1977). A detailed analysis of sedimentation rates at Gubbio suggests that the Maastrichtian — Danian faunal overturn might have happened very rapidly, of the order of 10,000 years or less (Kent *Geology* **7**, 66; 1979). Palaeontological work on a comparably complete sequence of pelagic deposits at Zumaya, Spain suggests that environmental stresses were first felt by planktonic foraminifera about 10,000 years before the end of the Maastrichtian, following which occurred the major crisis involving extinction of globotruncanid foraminifera and a drastic change in the nannoplankton flora (Percival & Fischer *Evol. Theory* **2**, 1; 1977).

Interest naturally focuses on whether or not the dinosaur extinction was coincident in time with that of the planktonic groups. Only if it was precisely coincident can the case for a drastic extra-terrestrial event seriously be upheld. Butler *et al.* (*Nature* **267**, 318, 1977) undertook a magnetostratigraphic study of a sequence of terrestrial sediments in New Mexico which they argued exhibited an unbroken transition across the Cretaceous — Tertiary boundary. By correlating with the Gubbio sequence they inferred that the dinosaurs became extinct slightly later than the planktonic foraminifera, perhaps by as much as half a million years. This correlation has been criticised by Alvarez *et al.* (*Geology* **7**, 66; 1979) who suggest that an unconformity might be present in the New Mexico sequence. If so, the marine and terrestrial extinction events could indeed be synchronous. Further information on the issue has recently been published by Lerbekmo *et al.* (*Nature* **279**, 26; 1979) in another magnetostratigraphic study of a terrestrial sequence in Alberta. Their data indicate an approximate synchroneity of dinosaur and foram extinctions just below anomaly 29, the maximum error being about 100,000 years.

Oxygen isotope data from foraminifera in deep sea cores has been used to monitor water temperature changes across the Cretaceous — Tertiary boundary. McLean (*Science* **201**, 401; 1978) cited isotope work on benthic forams indicating a late Maastrichtian warming event and went on to propose a novel interpretation of dinosaur extinctions by a temperature rise rather than a temperature fall, as had been frequently speculated previously. He failed, however, to cite other isotope work indicating just the reverse, a slight cooling through Maastrichtian time (Savin & Douglas *Bull. geol. Soc. Am.* **84**, 2327; 1973; Saito & Van Donk *Micropalaeontology* **20**, 152; 1974). This latter interpretation is indeed more in accord with what is known from the terrestrial plant record (for example Krassilov *Palaeogeog., Palaeoclimatol., Palaeoecol.* **17**, 157; 1975).

The most recent isotope data is reported from an exceptionally well documented Atlantic core by Thierstein and Berger (*Nature* **276**, 461; 1978). The oxygen isotope values remain more or less stable through the Maastrichtian part of the core and then exhibit a sharp change into the basal Danian. This can be interpreted as signifying a sudden water temperature increase (in which case it comes too late to support McLean) or as a salinity decrease. Thierstein and Berger favour the latter interpretation and cite the evidence as support for their hypothesis that the extinction event was caused by a large-scale invasion of the ocean by low salinity water from a previously isolated high latitude basin. Their completely original idea on mass extinctions is likely to prove as controversial as many others on this subject, especially as the existence of extensive high latitude ice masses at the end of the Cretaceous seems unlikely for a number of reasons, and it is hard to see how otherwise the requisite large volume of low salinity water could be created. Despite all the new data and ideas it seems probable that the extinctions at the end of the Cretaceous will continue to intrigue us for some time to come. ☐

SEASAT's short-lived mission

from T. D. Allan

On 10 October, 1978, after little more than one hundred days of operation, the transmissions from SEASAT-1 — the first satellite dedicated to a study of the ocean surface — were brought to an untimely end by a catastrophic short circuit in the power system.

The declared first objective of SEASAT's mission was to test whether satellite microwave sensors could provide data of a high enough accuracy to be incorporated into existing systems for forecasting and charting global ocean conditions. It was an ambitious project which had been planned for over 10 years by oceanographers and NASA engineers. The accuracy criteria laid down as design goals for the satellite sensors fell little short of the accuracies that can presently be achieved by ships and surface buoys. If SEASAT's sensors can be shown to have achieved these precisions under different ocean conditions then routine monitoring of the sea surface from satellites becomes a real and exciting alternative to the sparse data of variable quality currently obtainable from surface vessels.

The major difference in SEASAT's payload from previous environmental satellites was the concentration of radar sensors with the all-weather capability that is essential for repetitive surveys of sea areas mostly covered by cloud. Of the four microwave sensors carried on SEASAT — three active and one passive — the radar altimeter had some space heritage, previous versions having been flown on Skylab and GEOS-3. However, no instrument had even measured its own orbiting height to an accuracy of ± 10 cm which was the precision required to provide useful information on ocean currents, tides and surface waves.

The radar scatterometer was designed to satisfy the weather forecasters' requirements for a comparatively dense grid of wind field observations. The instrument swept out swathes of over 500 km on either side of the spacecraft's sub-orbital track and related ocean backscatter to surface winds. Further information on wind speed was provided by the passive microwave radiometer which measured brightness temperature at five frequencies in the range 6.6 — 37 GHz.

The most controversial of SEASAT's suite of sensors was the synthetic aperture radar (SAR) which, operating at 1.3 GHz (23 cm wavelength), was designed to produce an image of the sea surface with a spatial resolution of 25 m. The data rate required to achieve such a fine resolution is so high (approximately 100 megabits s^{-1}) that on-board recording was out of the question and SAR could only be operated in real time over areas within range of a ground receiving station. The European SAR station was at Oakhanger in Hampshire, operated by the Royal Aircraft

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T. D. Allan is Chairman, Seasat Users Research Group of Europe.

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Establishment (Farnborough) under an agreement between ESA and NASA.

The performance of a satellite's sensors must be assessed against reliable surface observations and the question now being asked of the SEASAT mission is whether sufficiently diverse surface data were obtained last summer to satisfy the main 'proof-of-concept' objective. A special data-gathering cruise was organised by the US National Oceanographic and Atmospheric Administration (NOAA) in the Gulf of Alaska during a 4-week period in August-September 1978. Observations of wind, waves, temperature and other parameters were made by two ships, four aircraft and an array of surface buoys. A detailed comparison between these observations and the signals from SEASAT's sensors was made recently during two workshops held at the Jet Propulsion Laboratory, Pasadena. More than 60 scientists and engineers from several oceanographic and space research centres were involved and the preliminary results for each sensor have been reported in *Science* (**204**, 1405-1424; 1979).

The scatterometer task group had evolved three candidate algorithms which made slightly different assumptions in relating the measured scattering coefficient to the wind vector as a function of incidence angle, azimuth angle and polarisation. Since these were largely constructed from a limited set of aircraft measurements it is not surprising that the satellite models required some modifications especially with regard to determining wind direction unambiguously. An analysis of some 30 comparisons showed the scatterometer winds to be biased high by 1.9 m s^{-1} with a standard deviation about this bias of 1.8 m s^{-1}. When the scatterometer values were compared with wind fields produced by kinematic analysis techniques (as opposed to spot measurements) wind speed differences were in the range 1.1–5.2 m s^{-1} (scatterometer higher) and for direction the means were less than 10° with standard deviations of approximately 20°. Wind speeds were generally low throughout the experiment and part of the observed differences may be attributed to errors in the surface spot observations.

The scanning microwave multi-channel radiometer (SMMR) measured Earth's radiation at five microwave frequencies at both vertical and horizontal polarisation. The algorithms to extract sea surface temperature and wind speed are correspondingly complex since they involve ten values of brightness temperature with different weightings according to frequency, to the antenna pattern, and to the amount of cloud and rain present. Over open ocean the best wind speed determinations gave a standard deviation of 3 m s^{-1} about a bias of 1.5 m s^{-1}. Sea surface temperature estimates were disappointing with a standard deviation of 1.5 °C about a bias of 3-5 °C. However, further analysis may determine the cause of the bias and suitable correction can be made. The SST value is averaged over a footprint approximately 120 km on the side.

In validating the performance of the radar altimeter the GOASEX data were used to calibrate only significant wave height and radar backscatter; the calibration of the altimeter's height determination was made over a range in Bermuda. During its last few weeks of operation SEASAT was manoeuvred into a frozen orbit pattern which allowed it to pass over Bermuda every 3 days. The calibration range included high precision tide gauges and a laser tracking system. For four overhead passes where laser tracking was possible the weighted mean bias in the altimeter-derived height was -50 ± 11 cm (over a height of 800 km). During the engineering assessment of the altimeter the noise in the height measurements for a 4-m significant wave height was 5-8 cm. If relative changes of this order can be detected in sea surface topography than valuable information can be obtained on ocean circulation patterns, eddies, tides, and the marine geoid. For this reason, the altimeter represents perhaps the greatest loss to the oceanographic community.

There was some lively discussion before the synthetic aperture radar was flown on SEASAT as to its ability to image surface waves. SAR sweeps out a 100 km swathe centred about a point 250 km to the right of the satellite's sub-orbital point. Each target remains in the radar beam for about 2 seconds and it was argued that in this time the movement of the shorter capillary waves which scatter the L-band energy could be more than enough to blur the return image. And since the detection of the larger gravity waves was seen to depend on their modulation of the shorter waves, it seemed doubtful if they could be detected. In the event, SAR produced several images of swell waves not only in the Gulf of Alaska but in other ocean areas including those covered by the Oakhanger station. Wave directions can be estimated to a few degrees and wave length greater than about 100 m appear to show clearly, given a certain minimum significant wave height (apparently around 2 m), certain minimum wind conditions and, up to the present, a wave direction predominantly across the satellite track. Apart from imagery of surface swell the SAR record analysed to date has shown remarkable examples of internal wave trains, surface pollutants, ice features in the Arctic, and details of shallow sand banks in the Channel and other shallow water areas around the UK. Its fine resolution (25 m against Landsat's 80 m) and its independence from adverse weather conditions have now aroused the interest of land surveyors and geologists.

Before the launch of the spacecraft the SEASAT Users Research Group of Europe (SURGE) had submitted a list of proposed experiments to NASA and as a result SAR was activated over a number of European sites. The most important of these was JASIN — a Joint Air-Sea Interaction experiment in a comparatively small area close to Rockall in which up to 14 research vessels, 3 aircraft and 35 buoys took measurements in the period mid-July to mid-September. Although SEASAT's early demise is proving inceasingly regrettable as its results become available, it was a piece of extremely good fortune that one of the most intensive research programmes of sea-surface observations took place during its short life. Now that the sensor algorithms have been refined and modified following GOASEX, the JASIN data should allow a comprehensive assessment of the performance of each microwave sensor. It may still be possible to go a long way towards reaching SEASAT's first objective — to prove the concept of reliable remote sensing of the seas from satellites. □

100 years ago

It may interest our readers to know the elevations which at present are reached by lines of railway in different parts of the world. The Apennine Railway reaches its highest point at an elevation of 617 metres above sea-level; the Black Forest Railway ascends to 850 metres, the Semmering line to 890, the Caucasus line to 975 metres. The St. Gothard tunnel is 1,154 metres above sea-level; the railway across the Brenner reaches 1,367 metres; the Mont Cenis Railway ascends to 1,338 metres, the North-Pacific line to 1,652, the Central-Pacific to 2,140, and the Union-Pacific to 2,513 metres. The highest of all is the line across the Andes, which reaches an elevation of 4,769 metres.

We have on our table the following works: — "The Spiders of Dorset," Rev. O. Pickard Cambridge; "Chemical and Geological Essays," by T. Sterry Hunt (Trübner); "Deaths in Childbed," Dr. Æneas Munro (Smith, Elder, and Co.); "The Silk Goods of America," W.C. Wyckoff; "Structural Botany," Dr. Asa Gray (Trübner); "Luxurious Bathing," A. W. Tuer (Field and Tuer); "Phrepology Vindicated," A. L. Vago (Simpkins); "On the Diffusion of Liquids," J. H. Long (H. Laupp); "Reform Essays on Incentive Religion and Warfare;" "Farming for Pleasure and Profit" (Poultry Keeping), Arthur Roland (Chapman and Hall); "Manual of Practical Anatomy," J. Cossar-Ewart (Smith, Elder, and Co.); "Rays from the Realms of Nature," Rev. James Neil (Cassell); "Jack's Education; or, How He Learnt Farming," Prof. Henry Tanner (Chapman and Hall); "Vocal Physiology and Hygiene," Gordon Holmes (Churchill); "Fauna der Gaskohle und der Kalksteine der Perm Formation Böhmens," Dr. Ant. Fritsch.

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review article

Incommensurable structures

R. Pynn

Institut Laue-Langevin, 156X Centre de Tri, 38042 Grenoble Cedex France

The discovery of incommensurably distorted structures has important implications for the study of structural phase transitions.

ON 8 June, 1912 von Laue and coworkers presented a paper entitled *Interferenz-Erscheinungen bei Roentgenstrahlen* in which a theory of the diffraction of X rays by a periodic lattice of atoms was outlined[1]. In 1913, Bragg[2] reported the structures of four alkali halides determined by X-ray techniques. Since these early experiments many thousands of crystal structures have been determined and the concepts of crystal periodicity and symmetry have had a central role in the development of solid state physics. According to the conventional view crystalline solids are composed of plane-sided cells which are stacked on a regular lattice of sites. Depending on the symmetry of the lattice and of the arrangement of the atoms within each cell, a crystal is assigned to one of the 230 possible space-symmetry groups. By definition, when any of the symmetry elements which comprise the space group are applied to the crystal it is mapped into itself and its physical properties are unchanged. Apart from some pathological and (as we shall see) interesting exceptions the symmetry groups encountered in practice comprise rotations, reflections and translations in our three-dimensional world. Nevertheless, it is simpler, and more relevant to this article, to consider a one-dimensional crystal (chain) such as that depicted in Fig. 1*A*. Evidently, left–right translation of the chain by any multiple of the unit-cell side, *a*, leaves the chain unaltered, as does inversion (reflection) through a suitably chosen point.

Structural transformations

Many solid materials have been found to change their crystal structure when some externally applied perturbation such as temperature is varied. Structural transitions of this type, particularly those which occur continuously, have been the subject of intensive study during the past 20 years or so[3]. One type of continuous transition of the so-called displacive type, can be visualised with the aid of Fig. 1. Suppose that the atoms of the chain are subjected to longitudinal (left–right) displacements whose amplitudes vary sinusoidally in space as shown in Fig. 1*B*. The atomic positions which result from this modulation will be as depicted in Fig. 1*C*. A transition between the distorted chain and the high-symmetry uniform chain occurs when the modulation amplitude vanishes. This amplitude is thus the order parameter of the transition and plays an analogous role, for example, to the magnetisation in a transition between ferromagnetic and paramagnetic states. The fact that the modulation vanishes continuously at the transition temperature, T_c, gives rise to the designation 'continuous transition'.

Much of the detailed information which has been obtained about real structural transformations has been derived from diffraction experiments with X rays or neutrons[4]. Once again, these experiments may be understood with the aid of a one-dimensional example. Suppose that a beam of X rays of wave vector $\mathbf{k}_0$ (wavelength $\lambda_0 = 2\pi/k_0$) impinges on the one-dimensional chain depicted in Fig. 2*A*. Each atom of the chain acts as a scattering centre and radiates X rays of wave vector $\mathbf{k}$ where $|\mathbf{k}| = |\mathbf{k}_0|$. The interference between the various scattered beams will be constructive only if the component of $\mathbf{k}_0 - \mathbf{k}$ in the *y*-direction is a multiple of $2\pi/a$. Generally, the quantity $\mathbf{k}_0 - \mathbf{k}$ is known as the scattering vector and is denoted by $\mathbf{Q}$. In the reciprocal space defined by the cartesian components of $\mathbf{Q}$, diffraction from the one-dimensional chain occurs only for $Q_y = 2\pi n/a$ where n is an integer. The values of $\mathbf{Q}$-components (Q_x, Q_z) perpendicular to the chain are immaterial. Thus the loci of allowed scattering vectors comprise a series of sheets in reciprocal space, as shown in Fig. 2*B*. These arguments may be easily extended to both two- and three-dimensional scattering arrays. A two-dimensional array (Fig. 2*C*) may be regarded as a chain (in the *x*-direction) of chains (in the *y*-direction). Its diffraction pattern is therefore characterised by the intersections of two mutually perpendicular sets of planes in reciprocal space. As shown in Fig. 2*D*, this gives rise to rods of scattered intensity. Extending the argument to one more dimension one finds that the diffraction pattern of a three-dimensional crystal is a three-dimensional array of points in reciprocal space. While such an array of Bragg points is the most commonly observed form of diffraction, patterns similar to those depicted in Fig. 2 are not simply figments of the imagination, but can (as will be shown) be observed in the laboratory.

As we have seen, the diffraction pattern of the uniform chain in Fig. 1*A* consists of a series of sheets in reciprocal space separated by multiples of $2\pi/a$. The diffraction pattern of the modulated chain (Fig. 1*C*) may be deduced in an analogous manner. As a result of the choice of the modulation wavelength as $4a$, every fourth atom is displaced by the same amount. The distorted structure can thus be described in terms of a unit cell which is four times larger and contains four times as many atoms as the original one. Accordingly, the diffraction pattern of the distorted phase consists of sheets separated by $\pi/2a$ rather than by $2\pi/a$. That is, three equally spaced sheets are inserted between each pair shown in Fig. 2*B*. Evidently, the intermediate sheets, known as satellite reflections, disappear when the amplitude of the lattice modulation tends to zero. (When all sheets are equally spaced, the intermediate ones are generally known as superlattice reflections. The term 'satellite reflection' is used here to avoid a proliferation of terminology.) Thus, the intensities of the satellite reflections serve as a measure of the modulation amplitude, that is, of the order parameter of the transition.

The previous example is a prototype for most of the continuous structural transitions which have been studied by diffraction

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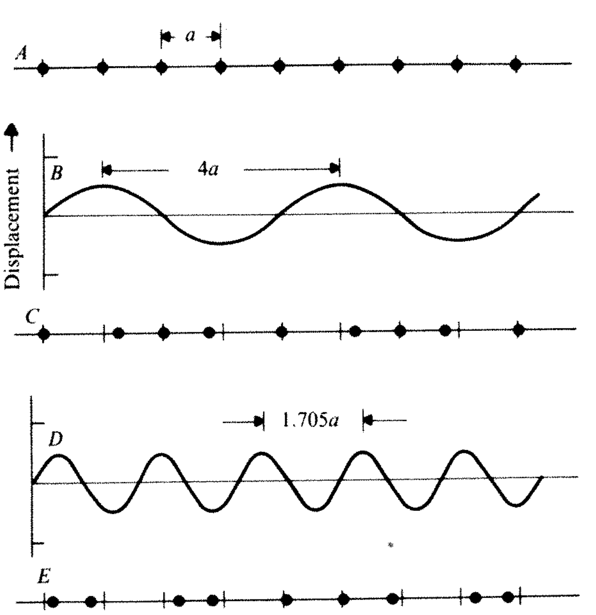


Fig. 1 *A*, a uniform chain of atoms; *B*, a commensurable modulation; *C*, distorted chain which results when the modulation of *B* is imposed on the chain in *A*; *D*, an incommensurable modulation; *E*, distorted chain which results when the modulation of *D* is imposed on the chain in *A*. In rows *A*, *C* and *E* the tick marks represent the positions of the undisplaced atoms.

techniques during the past 15 years or so. In general, the modulations which have been observed apply to a three-dimensional crystal and the diffraction patterns consist of a lattice of points rather than sheets in reciprocal space. Nevertheless, the distinction between main Bragg points and satellite reflections remains the same. The canonical example[5] of this type of phase transition is that which occurs at 105 K in $SrTiO_3$. Above the transition temperature the crystal structure, shown in Fig. 3*B*, consists of Ti atoms at the corners of a cubic cell surrounding a Sr atom at the centre of the cell. Each Ti is surrounded by oxygen atoms located at the apeces of the octahedra drawn in Fig. 3*B*. In the low-temperature (distorted) phase the oxygen octahedra, which are linked by a common oxygen atom at their corners, are rotated in the senses indicated by the arrows. This cog-wheel type of rotation is shown more clearly in Fig. 3*A*, where the positions of the oxygen atoms at low temperature are indicated by circles while their positions above the transition are indicated by hatched circles. Evidently, the low temperature distortion is periodic but the modulated variable is the angle of rotation of the octahedra rather than the longitudinal atomic displacement of Fig. 1. The periodicity of the modulation (see Fig. 3) is, however, enough to cause a doubling of each unit-cell side.

Most previous reports on structural transitions described systems in which the unit-cell size either remained the same or was changed by a simple multiple at the transition. However, it is certainly not *a priori* necessary for a lattice modulation to have a periodicity which is a rational multiple of a unit-cell side. An example of a modulation which does not satisfy this simple criterion and which is thus incommensurable with the undistorted lattice is shown in Fig. 1*D*. (The term incommensurate has been used in this context, probably without malice. Incommensurate is defined as not adequate.) The atomic arrangement in the incommensurable phase is depicted in Fig. 1*E*. This phase has the remarkable property that no two atoms are displaced by the same distance from their positions in the undistorted phase. It is thus impossible to find any lattice translation which maps the crystal into itself. For the case of a three-dimensional crystal this means that the crystal does not belong to any of the 230 space groups mentioned earlier. In spite of this apparent lack of symmetry, the diffraction pattern of an incommensurably modulated structure is relatively simple. Just as for a commensurable distortion there are principal reflections, separated by $2\pi/a$, and satellites, whose positions depend on the wave vector q_0 of the modulation ($q_0 = 2\pi/1.705a$ for Fig. 1*D*, *E*). The satellites are separated from the principal reflections by multiples of q_0.

Symmetry classifications of incommensurably modulated structures have been developed[6]. The distorted chain in Fig. 1*E* is mapped into itself by an operation which translates the chain to the right by a distance na and changes the phase of the modulation by q_0na. Thus, the translational-symmetry group of the crystal consists of operations which translate the crystal and, at the same time, slide the distortion wave with respect to the crystal. This means that the symmetry operations act in a hyperspace whose dimensionality is greater than that of the crystal. For a three-dimensional crystal with a single modulation wave, this hyperspace is four-dimensional and comprises the three spatial dimensions and a dimension which refers to the phase of the modulation.

Incommensurable phase transitions

The first structurally incommensurable phase transition[7] to be identified as such was found in the insulator $NaNO_2$. Subsequently, such transitions have been discovered both in metallic and insulating materials. The physical effects which drive the transitions are somewhat different in the two cases. For the insulators it is often found that competing, short-range interatomic forces of different ranges and similar magnitudes give rise to an incommensurably modulated phase. To see crudely how this may arise, consider the monoatomic chain in Fig. 1 and suppose that only elastic forces between first and second neighbour atoms contribute to the energy. The latter may then be written as

$$U = \sum_{n=1}^{N} \left\{ A_1 \sum_{i=\pm 1} (u_n - u_{n+i})^2 + A_2 \sum_{j=\pm 2} (u_n - u_{n+i})^2 \right\} \quad (1)$$

where u_n is the longitudinal displacement of the nth atom from its lattice site and A_1 and A_2 are the first and second neighbour harmonic force constants, respectively.

Expanding the displacements as a Fourier series

$$u_n = \sum_q u_q \cos qna \quad (2)$$

and substituting in equation (1) one finds that the energy is given by

$$\begin{aligned} U &= 4 \sum_q u_q^2 [A_1(1 - \cos qa) + A_2(1 - \cos 2qa)] \quad (3) \\ &= 4 \sum_q u_q^2 A_q \end{aligned}$$

A sinusoidally modulated structure will be stable if A_q in equation (3) has a minimum value for a non-zero wave vector q_0. Necessary conditions for this to occur are that

$$\frac{\partial A_q}{\partial q} = 0; \quad \frac{\partial^2 A_q}{\partial q^2} > 0 \quad (4)$$

at the wave vector q_0. These conditions imply that a modulation of wave vector q_0 given by

$$\cos q_0 a = -A_1/4A_2 \quad (5)$$

will be stable provided $A_1 < 4|A_2|$ and $A_2 \leq 0$.

A well-studied system which exemplifies the above calculation is the insulator K_2SeO_4. In this material[8], which transforms to an incommensurable phase at 130 K, force constants (see A_1 and A_2 above) between first- and second-neighbour layers of atoms decrease strongly with decreasing temperature above 130 K. However, third-neighbour interlayer forces increase slightly in the same temperature interval. It is thought that the competition between these short-range forces is responsible for the observed transition.

For conducting materials, interesting incommensurable transitions result from the interaction between conduction electrons and the atomic lattice. These interactions give rise to effective interatomic forces which are long-range. Although the appearance of an incommensurable phase could be described in terms of competition between such forces, this language is unusual for metals. Rather, attention is focused on the fact that in certain cases the energy of a metal is lowered if the conduction-electron density is spatially modulated[9,10]. Thus, a so-called charge density wave (CDW), whose wave vector is an extremal Fermi surface dimension, is set up in the conduction electron gas. The ions of the lattice respond to the CDW, in an attempt to preserve local charge neutrality, and an incommensurable lattice modulation is thereby generated. Examples of this phenomenon are to be found among the transition metal dichalcogenides[11], the one-dimensional charge-transfer salts (such as TTF–TCNQ[12]) and certain chain compounds (for example KCP[13]). The transition to the incommensurable phase is known as a Peierls transition.

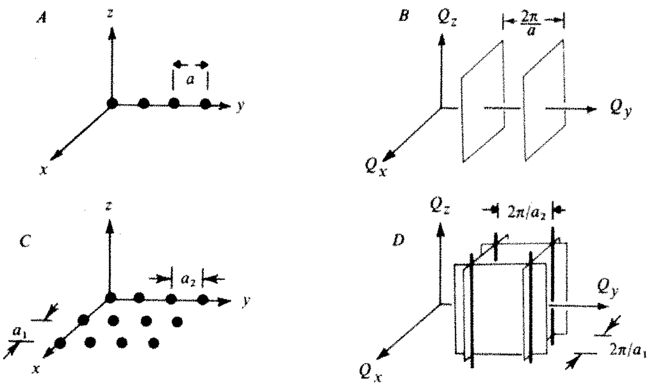
Anharmonic effects

Although instructive, the calculation leading to equation (5) is deficient in several ways. First, equation (1) is a truncated expansion of the total energy in terms of the displacements u_n. There ought to be terms of higher order in u_n. These so-called anharmonic terms limit the magnitude of the amplitude of the incommensurable distortion which, according to equation (3), is otherwise unbounded. A second defect of equation (1) is that it is a zero-temperature approximation. At finite temperatures, the addition of the obligatory entropy term may radically alter the behaviour deduced above, and may even suppress the distorted phase entirely.

In addition to restricting the amplitude of a modulation, the anharmonic terms mentioned above have an important qualitative effect on the nature of a modulated phase. In a commensurably modulated structure, such as Fig. 1*C*, the anharmonic terms determine the optimum phase of the distortion (Fig. 1*B*) with respect to the uniform lattice (Fig. 1*A*). For a particular choice of phase, such as that depicted in Fig. 1*A*, *B*, in which every other atom is undisplaced in the modulated phase, the energy is minimised. This situation is unique to commensurably modulated structures. In the incommensurable case, the phase of the distortion is, by definition, different at each lattice site. The energy of the system is thus unchanged if the modulation is translated with respect to the lattice and the concept of 'optimum phase' has no meaning. Nevertheless, in any small region, the incommensurable wave of Fig. 1*D* causes a distortion which is very similar to that of a commensurable modulation of wavelength $5a/3$. There are even regions in which the distortion has a phase which reduces the local contribution to the energy. However, these regions are compensated by others in which the distortion has the 'wrong' phase and the local energy is large. In this situation there are two ways in which the sinusoidal modulation may be modified in order to lower the energy of (render more stable) the distorted phase. First, the amplitude of the distortion may be increased in the 'correct'-phase regions and decreased in the 'wrong'-phase regions. Alternatively, the phase may be distorted and made to vary from region to region. It is generally accepted that the latter possibility occurs most readily. To calculate the effect[14] it is necessary to return to equation (2) and to replace the discrete atomic positions (na) by a continuous coordinate x. Equation (2) is then rewritten as

$$u(x) = A\cos(q_c x + \phi(x)) \tag{6}$$

where q_c is a commensurable wave vector close to the wave vector of the sinusoidal distortion deduced from equation (5). $\phi(x)$ is a phase whose variation with position, x, minimises the energy obtained when equation (6) is substituted in the anharmonic (non-truncated) version of equation (1). The equation which results from this minimisation procedure is known as the (static) Sine–Gordon equation[15]. This nonlinear differential equation has solutions similar to those represented by the stepped curve in Fig. 4. The straight line in this figure represents the phase variation which would correspond to the simple, sinusoidal modulation with a wave vector given by equation (5). Each step in the curve in Fig. 4 represents a sudden change of phase of the modulation. The steps can be regarded as walls separating domains in which $\phi(x)$ is independent of x and which are thus commensurably distorted. In these domains the 'optimum phase' argument applies and the anharmonic energy is thereby minimised. This reduction of energy of the system more than offsets the cost in harmonic energy which results because $\phi(x)$ does not follow the straight line in Fig. 4, but is stepped instead. Evidence for the importance of anharmonic effects and for the plausibility of the solution sketched in Fig. 4 have been obtained, for example, by Moncton *et al.*[16] in their neutron scattering experiments with 2H–TaSe$_2$.



Direct space Reciprocal space

Fig. 2 Atomic arrays in direct space (*A*, *C*) and their associated diffraction patterns in reciprocal space (*B*, *D*).

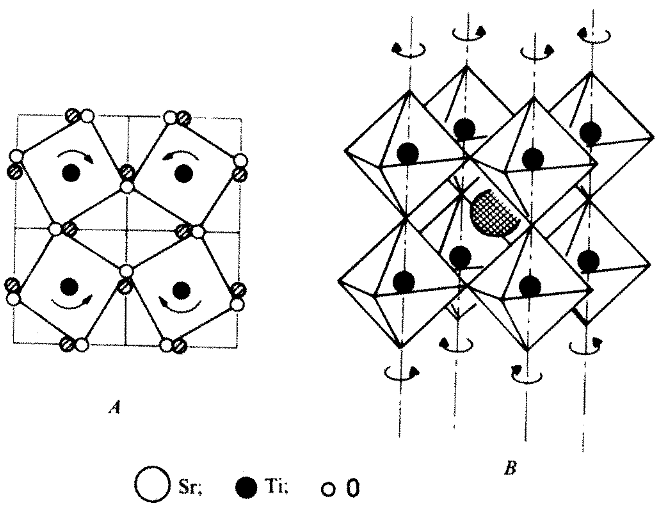


Fig. 3 *A*, A plan view of the structure of SrTiO$_3$ below the 105 K phase transition. The hatched circles represent the positions of the oxygen atoms in the high temperature (symmetric) phase. Arrows indicate the 'cog-wheel' type distortion induced by the transition. *B*, Three-dimensional representation of the symmetric phase of SrTiO$_3$.

The phase steps in Fig. 4 are called solitons. They are an example of the solitary waves or kinks which are the solutions to many nonlinear wave equations. As an aside, one may note that solitary waves are not a new phenomenon in physics. In 1844, Russell reported a solitary water wave which he had observed in a canal[17]: "... rolled forward with great velocity, assuming the form of a solitary elevation, a rounded, smooth and well-defined heap of water, which continued its course along the channel apparently without change of form or diminution of speed. I followed it on horseback ... and after a chase of one or two miles I lost it in the windings of the channel". The solitary wave pursued by Russell bears a singular resemblance to the derivative $d\phi/dx$ of the function plotted in Fig. 4 although, in fact,

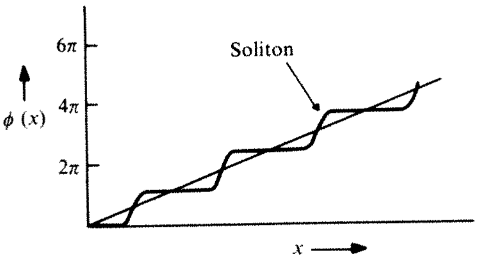


Fig. 4 Variation of the phase $\phi(x)$ (see equation (6)) in an incommensurably modulated structure.

the equations governing the two phenomena are not quite the same. (The relevant equation in this case is known as the Korteweg–de Vries equation.)

Figure 4 shows that the difference between a commensurably distorted phase and an incommensurably distorted one is that the latter contains a finite number of solitons. In many cases, it is found that the intersoliton spacing increases as the temperature of an incommensurably modulated phase is decreased. Finally, the number of solitons may become zero at a transition to a commensurably distorted structure. Such a transition, examples of which have been found in both metallic[12] and insulating systems[8], has become known as a lock-in transition. At the transition, the periodicity of the distortion 'locks in' to that of the underlying lattice.

Fluctuations

The picture presented so far of an incommensurably distorted structure has been a static one. Nevertheless, at finite temperatures there will be fluctuations of atomic positions about their mean values. As always, these fluctuations can be Fourier analysed in both space and time and each Fourier component represents a normal mode or phonon. In view of the somewhat tangled atomic arrangement which is to be expected in the domain walls (solitons) this Fourier analysis probably involves many coupled terms. It may therefore be more profitable to think about possible fluctuations of the distortion wave (see equation (6)) itself. Two fluctuation modes suggest themselves: phase fluctuations (variations of ϕ with space and time), and amplitude fluctuations (variations of A). The first were originally discussed by Overhauser[18] who suggested the name phason for these modes which consist, in fact, of fluctuations in the positions of the solitons. With lack of originality I (and perhaps others?) adopted the name amplitudon for the second type of mode. Not surprisingly, the ability to name these modes has not greatly accelerated their discovery and, at the time of writing, there has been no unambiguous identification of a phason made in an incommensurably modulated phase.

Phasons have been found[19], however, in the unusual chain compound $Hg_{3-\delta}AsF_6$. In this material, linear chains of mercury cations are imbedded in a tetragonal matrix of AsF_6^- anions. The inter-mercury spacing is incommensurable with the lattice constant of the matrix and the (long wavelength) phasons consist of rigid motion of the chains with respect to the matrix. At sufficiently high temperatures (above 120 K) the mercury chains are uncorrelated and, in a neutron diffraction experiment, produce sheets of scattered intensity similar to those in Fig. 2*B*. Phasons are observed around these diffuse sheets.

Surfaces

In the above discussion, the word incommensurable referred to the relationship between the periodicities of a basic structure and of the modulation which distorts it. However, the word is also used in a slightly different context, which is well exemplified by the case of a rare gas monolayer on a crystalline substrate. A one-dimensional representation of this system is displayed in Fig. 5. The period which the lattice of gas atoms would adopt in the absence of the substrate is not the same as the period of substrate atoms. The two periodicities are incommensurable. Since the substrate lattice is fairly rigid the interaction between the substrate and the adsorbed gas atoms tends to modulate the positions of the latter. That is, the substrate tries to impose its periodicity on the adsorbate. The adsorbate is thus analogous to the atomic chain in Fig. 1*A*, and the substrate is a manifestation of the abstract 'imposed modulation' which was described earlier and which is depicted in Fig. 1*D*. In view of the similarity between the substrate–adsorbate problem and the modulated-lattice problem, it is not surprising that many features of the solutions of these problems resemble one another. Neutron diffraction studies have been carried out, for example, on a monolayer of argon adsorbed on the surface of graphite[20]. Since the diffraction pattern of the layer is a set of rods in reciprocal space (Fig. 2*C*, *D*), while that of the substrate comprises the familiar Bragg points, the sources of diffraction are distinguishable. In the argon–graphite system the adsorbate periodicity at low temperatures is incommensurable with that of the substrate. In such incommensurable layers a soliton (or domain wall) structure, similar to that discussed for the linear chain, and sketched in Fig. 5, is expected[21,22]. Transitions between commensurable and incommensurable monolayers have been studied by electron diffraction techniques by Fain[23,24], Venables[25] and their coworkers.

A phenomenon which occurs in adsorbate–substrate systems and which is related to incommensurability is orientational epitaxy[26,27]. This uninformative phrase applies to a situation in which the crystallographic (symmetry) axes of an incommensurable adsorbate are rotated slightly with respect to the axes of the substrate. Examples of this effect are found with silver adsorbed on mica[28] and with some rare gases (notably Ar[29]) adsorbed on graphite. The effect arises because the interaction energy of the substrate and the (incommensurable) adsorbate depends on the forces, represented by springs in Fig. 5, between the adsorbate atoms. Less energy is needed to produce a shear distortion of the adsorbate than is needed for a compression or contraction of similar magnitude. Thus, the shear is favoured and the adsorbate is rotated with respect to the substrate.

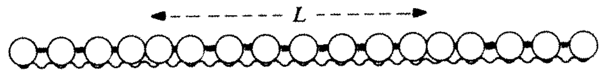


Fig. 5 Artist's view of interacting gas atoms adsorbed on the surface of a crystalline substrate. Two domain walls separated by a distance *L* are depicted. Between the walls, the gas atoms are in registry with the substrate.

Conclusions

I have attempted to review simply some of the underlying ideas involved in the study of incommensurable systems. Since the subject is still developing, this article must soon be obsolete. Nevertheless, the discovery and study of incommensurably distorted structures is a milestone in the investigation of structural phase transitions and deserves recording. The explicit inclusion of nonlinear effects such as solitons marks a small but significant step away from the traditional treatment of harmonic or weakly anharmonic systems.

Linear atomic chains have been used to illustrate various phenomena for three reasons. First, it is pedagogically simpler. Second, and purely pragmatically, it is only in one dimension that exact solutions to nonlinear problems, such as the Sine-Gordon equation, are available. Third, and of most interest, many of the systems which are incommensurably modulated are one-dimensional in some sense. This applies particularly to the so-called one-dimensional conducting materials, such as TTF–TCNQ, which have received so much publicity during the past 5 years. In these systems, the observation of diffraction patterns similar to that in Fig. 2*B* is convincing evidence that one-dimensional physics is relevant to the real world.

I thank J. D. Axe, P. C. Hemmer and T. Riste for their critical comments.

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articles

The structure of the radio jets in 3C449

R. A. Perley
National Radio Astronomy Observatory, VLA Program, Socorro, New Mexico 87801
A. G. Willis
Department of Physics, Brandeis University, Waltham, Massachusetts 02154
J. S. Scott
Astronomy Program, University of Maryland, College Park, Maryland 20742

High resolution observations of 3C449 show the presence of two highly collimated symmetric jets on each side of an unresolved core. The jets show considerable fine structure and polarisation. Measurements of the depolarisation suggest internal densities of $\sim 0.02\ \mathrm{cm}^{-3}$ and flow velocities of $\gtrsim 1{,}000\ \mathrm{km\ s}^{-1}$.

INCREASING numbers of low-luminosity radio galaxies have been found to contain highly collimated radio jets which connect the central compact nuclear source to the distant outer lobes. Examples of one-sided jets have been found in radio sources such as NGC6251 (refs 1, 2) and 3C219 (ref. 3) as well as double-sided jets such as found in NGC315 (refs 4, 5) and 3C31 (ref. 6). These jets may be the pathways through which the outer lobes of radio galaxies are continuously supplied by fresh relativistic and thermal material from the central nucleus, although whether the jet is composed of separate components[7] or a beam[8] remains controversial. The potential importance of this class of radio source to theories of radio source structure and evolution has led to an extensive programme on the very large array (VLA) to map sources suspected of having radio jets. These observations are being reported elsewhere. Here we present high resolution VLA maps at 20 and 6 cm of the double-sided jet in the radio galaxy 3C449.

3C449 is identified with a cDE4 galaxy[9], the largest in a group of three (VV 6-49-29)[10]. Sandage[11] gives the redshift as $z = 0.0181$ and the visual magnitude as $m_V = 13.2$. With $H_0 = 75$ and $q_0 = 0.5$, 3C449 has a luminosity distance of 72 Mpc, and 1 arc s is equivalent to 340 pc linear extent.

3C449 is a member of the open cluster of galaxies Zw2231.2+3732, which itself is less than 30° from the edge of the Perseus supercluster[12], a system having the same redshift as 3C449.

The first interferometric maps showing the structure of 3C449 were made at 408 and 1,407 MHz[13]. More recent maps[14] at three frequencies made with the Westerbork array have clearly delineated the jet-like structure of this source. As the Westerbork observations did not resolve the jet itself, we have made higher resolution maps of the jet with the VLA at 20- and 6-cm wavelengths. Our new observations show that the jet has a highly complex structure.

Observations

The observations were made with the partially completed VLA. Twelve antennas were used, giving spacings from 250 m to 10 km. Eight of the antennas were placed on the south-west arm while the remaining four were on the south-east arm. Data on the array are given in Tables 1 and 2.

The data were phase and amplitude calibrated against 2200+420 (BL Lac) whose position was taken as $\alpha = 22\ \mathrm{h}\ 00\ \mathrm{min}\ 39.363\ \mathrm{s}$, $\delta = 42°\ 02'\ 08.57''$ (1950) (ref. 15). Three minutes out of every 15 were spent on the calibrator. The flux densities of 2,200+420 were found to be 2.95 ± 0.01 Jy and 3.05 ± 0.03 Jy at 4,885 MHz and 1,465 MHz respectively. These values were derived from simultaneous observations of 1328+307 (3C286), whose flux density at 4,885 MHz and 1,465 MHz was taken to be 7.41 Jy and 14.44 Jy respectively[16].

Because of the unbalanced distribution of antenna positions on the two arms (the south-west arm having antennas out to 10 km, the south-east arm only to 1.6 km) and the declination of

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Table 1 VLA systems parameters for observations of 3C449

Band (cm)	Frequency (MHz)	Bandwidth (MHz)	T_{sys}	Date (1978)	Polarisation
20	1,465	50	60°	29 September	Orthogonal linear
6	4,885	50	50°	1 October	Opposite circular

the source, the *u*, *v* plane coverage was non-uniform. In particular, a significant wedge in the *u*, *v* plane was not sampled. The resulting synthesised beam contained significant positive and negative side lobes, and the 'CLEAN' algorithm[17] was used to correct for their effects. Map restoration was done with a gaussian beam of the same half-power beamwidth and orientation as the synthesised beam.

As the shortest spacings at the time of our observations were ~250 m, some large scale structure is absent from our maps, particularly at 6 cm where the maximum fringe amplitude was ~40% of the total flux. Consequently, no 6-cm maps of structure beyond the jets were attempted. None of our maps have been corrected for the weighting due to the antenna power pattern. Since the FWHM of the primary beam at 6 and 20 cm are 9.0' and 30' respectively, these corrections will be quite small.

Structure of the jets

Figure 1 shows our full resolution 20-cm map with a beam size of 4.8 by 3.4 arc s in position angle (PA) −47°. Perhaps the most striking aspect of this source is the nearly perfect mirror symmetry of the jets and outer lobes about an axis through the central nucleus. Each jet is remarkably straight for some 50 arc s from the nucleus before deviating sharply towards the west, dropping in surface brightness and terminating in large diffuse lobes. As both jets bend in the same direction, rotation effects as a cause of this morphology are ruled out.

These large features continue the symmetry of the source; both bend back towards the east as the distance from the nucleus increases. Although both outer lobes are generally diffuse, several features are of interest. Both lobes are relaxed in appearance, which may suggest that they are in static equilibrium (thermally confined) with their surroundings (as opposed to dynamic or ram-confined equilibrium). In the northern lobe the western edge shows a much sharper rise in brightness than the eastern edge, and there seem to be two parallel ridges of emission orientated along PA ~45°. In the southern lobe, a ridge of emission is noticeable on the southeastern edge, roughly parallel to another ridge to the north-west. However, there is a significant chance that these ridges are artefacts of the CLEAN algorithm, as the Fourier components representing them will lie in the unsampled segment of the *u*, *v* plane. Beyond each lobe,

Table 2 Location of antennas

Antenna no.	VLA station no.	Distance from array centre (km)	Azimuth (deg)
1	W8	0.48	236
2	W32	5.22	236
3	E1	0.04	175
4	W16	1.59	236
6	W24	3.19	236
8	W40	7.66	236
9	W48	10.47	236
11	E8	.48	115
12	E15	1.59	115
13	E12	0.97	115
14	W14	1.26	236
15	W10	0.71	236

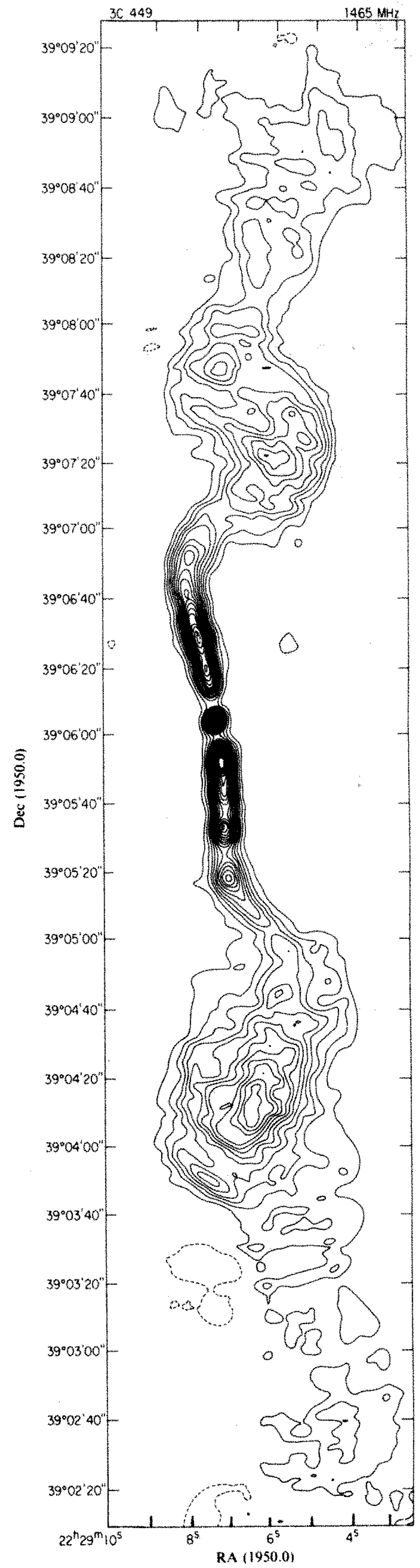

Fig. 1 3C449 at 1,465 MHz as observed by the VLA with a half-power beam of 4.8" × 3.4" in PA −47°. The contour interval is 5% of the peak brightness starting at +5%. The peak brightness on the map is 22.2 mJy per beam area and is located in the southern jet. The beam response is shown by the unresolved nuclear component.

low-brightness emission trails away beyond the edges of the map. A lower resolution map with a beam size of 15″ was made and showed the diffuse emission extending fully 15.8 arc min or 320 kpc with a slight overall curvature towards the west. The Westerbork 49-cm map of Bystedt and Högbom[14] shows low-brightness radio emission whose extension agrees with that seen in our low resolution map.

Returning to consideration of the morphology of the inner jets, we note: (1) within ~10 arc s (3.4 kpc) of the nucleus, both jets are greatly diminished in brightness, although some emission seems still to be present; (2) the southern jet exhibits higher brightness compact condensations. Similar enhancements may also be present in the northern jet although they are not so sharply delineated as the southern ones; (3) the two jets are not quite perfectly opposite each other, but intersect at an angle of 169 ± 2°.

A further map, made at 4,885 MHz, allows us to examine the fine scale structure of the jets. This map, having a resolution of 2.4″ × 1.0″ in PA −40°, is shown in Fig. 2. The high brightness condensations noted above at 20 cm are also apparent at 6 cm. The higher resolution shows that each jet is composed of numerous subpeaks or knots superimposed on a more general ridge of emission. Disregarding the inner 10 arc s low-brightness regions of the jets, straight lines drawn along the axes of the jets do not intersect at the nuclear source but at a point some 1.8 arc s north-west in PA −45°. Finally, the jets steadily widen with increasing distance from the nucleus, at a non-uniform rate. The greatest rate of expansion occurs within ~10–15 arc s from the nucleus where the surface brightness of the jets is minimal. At larger distances the opening angle seems approximately constant. Figure 3 shows the equivalent widths (total area under a cross-section profile divided by maximum height) of the jets measured for our 6-cm observations and corrected for beam convolution, as a function of distance from the nuclear source. Beyond distances of about 15 arc s from the nucleus, data for both jets are well fitted by a constant opening angle of ~7°.

The position of the unresolved nuclear source (< ~0.4 arc s in angular size) was found to be $\alpha = 22$ h 29 min 07.63 s ± 0.01 s, $\delta = 39°\,06'\,03.5'' \pm 0.1''$. Its flux density was 37 ± 1 mJy and 19 ± 1 mJy at 4,885 and 1,465 MHz; thus the nuclear source has a sharply inverted spectrum with $\alpha = -0.55$.

We compared the 6- and 20-cm maps to study spectral index variations along the jets; the results are shown in Fig. 4. The computations were made by comparing 6- and 20-cm maps made with similar resolutions. Although we are missing considerable flux from the 6-cm map due to missing short spacings, the jet brightness distribution, being a small scale feature, will be little affected. The missing structure will tend to make the spectrum even flatter than we have measured. In the main lobes, however, so much brightness is absent that no attempt was made to calculate the spectral index for these features. The average spectral index in the jets is 0.65 ± 0.1 in the north jet and 0.5 ± 0.1 ($S_\nu \propto \nu^{-\alpha}$) in the south jet. Some evidence for steepening of the spectrum is noted towards the ends of each jet although, curiously, the spectrum of the southern jet seems to be flattest at a distance of ~35 arc s from the nuclear source.

The jets are highly polarised at 6 cm, averaging ~30% along their lengths, although the southern one is somewhat less polarised than the northern one. A plot of the percentage polarisation and measured **E**-vector PA is shown in Fig. 5. The typical **E**-vector PA is 160° and changes rather little throughout the length of the jets. Haves[18] found the rotation measure of the integrated polarised flux to be -170 ± 4 rad m^{-2}. If this rotation measure is entirely external and applies to the jets, the intrinsic direction of the projected jet magnetic field is found to be 105°, that is, perpendicular to the jets to within our measurement errors. However, the polarised flux from the outer lobes must make a strong contribution to the total polarised flux and we must be wary of how representative Haves' rotation measure is in the vicinity of the jets, although a large negative rotation measure is characteristic of the galactic coordinates of 3C449 (ref. 19) ($l = 95°$, $b = -16°$). If our derived orientation of the projected magnetic field is correct, then the most likely intrinsic structure is that of a circumferential field.

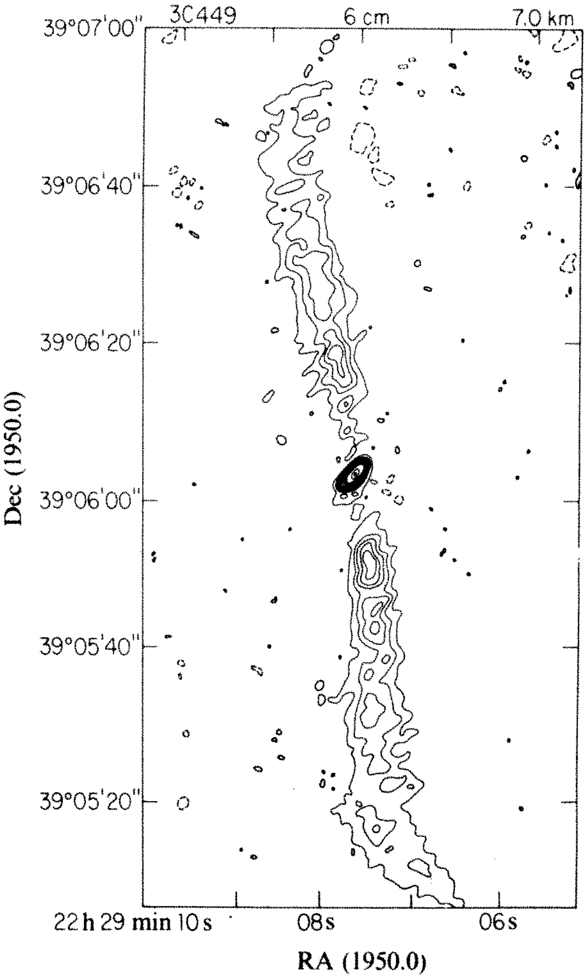

Fig. 2 The high resolution, 4,885 MHz map of the jets in 3C449. The synthesised beam is 2.4″ × 1.0″ in PA −40°. The contour interval is 1.5% of the peak brightness, starting at 1.5%, and the brightest contour is at 13.5% of the peak brightness, which is 37 mJy per beam area. The beam is shown by the nucleus, a 50% contour has been added to delineate the half-power beamwidth.

Discussion

We calculated equipartition parameters for the outer lobes and the jets using the standard formulae[20] but modified to derive the parameters directly from the measured integrated (over frequency) surface brightness, I, and path length through the emitting volume, s, rather than radio luminosity and source volume. The resulting field strength is given by

$$H_{\min} = \left[6\pi K \frac{I}{s} \frac{(1+k)}{|\sin\theta|^{3/2}}\right]^{2/7} \qquad (1)$$

and the total energy density in particles and fields is

$$\mu_{\min} = \frac{1}{24\pi}\left[6\pi K \frac{I}{s} \frac{(1+k)}{|\sin\theta|^{3/2}}\right]^{4/7} \qquad (2)$$

where k is the ratio of the energy in protons to electrons, and K is equal to $[c_2 c_{12}(\gamma)/c_1 c_3 c_8(\gamma)]$. The c-factors are defined and tabulated by Pacholczyk[20] and are functions of γ, the energy index of the assumed power law distribution of relativistic electrons. γ is related to the spectral index by $\gamma = 2\alpha + 1$. The angle θ is measured between the line of sight and the direction of the (assumed uniform) magnetic field.

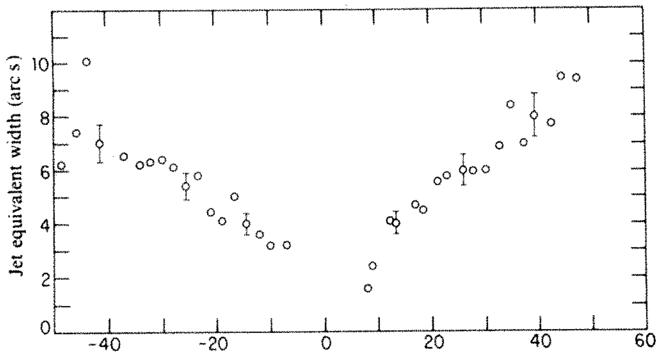

Fig. 3 The equivalent width of the jets found by planimetry of the 6-cm map shown in Fig. 3. The error bars shown are estimated from the noisiness of the corresponding profiles.

Our equations (1) and (2), combined with our 6- and 20-cm measurements of the intensity, give the magnetic field and total energy densities displayed in Table 3. The following assumptions have been made: (1) cylindrical geometry with the jet axis perpendicular to the line of sight, so that the path length through any feature is equal to the observed width of that feature. For the jets, we have taken the path length to be double the equivalent 6-cm width. This is appropriate for brightness distributions which are highly peaked at the centre (and is exactly true for a triangular brightness distribution). Our 6-cm profiles of the jet clearly show the brightness is in fact strongly peaked at the centre, and is not at all limb brightened, as may be expected for emission from a cylindrical sheath surrounding the jet. However, note that this observation is not conclusive evidence for emission from a filled cylinder, as emission from a circumferential magnetic field geometry (the configuration implied by the polarisation measurements) will appear centre brightened for virtually any distribution of the emitting gas due to the highly directive emissivity of synchrotron radiation. (2) The magnetic field lines are perpendicular to the line of sight through the centre of the feature, so that $\sin\theta = 1$. Note that if the field is purely circumferential, this requirement is automatically satisfied whatever the orientation of the jet may be, whereas if the magnetic field is longitudinal, our assumption requires the jet axis to be perpendicular to the line of sight. (3) The spectral index in the jet is 0.5, and 0.7 in the lobes. (4) The integration over frequency is from 10 MHz to 10 GHz. (5) Equal energies in relativistic electrons and protons ($k = 1$).

Note that the equipartition values of the internal energy density in the lobes ($\sim 5\times10^{-12}$ erg cm^{-3}) is approximately equal to the thermal particle energy density (nkT) in the intracluster medium of x-ray clusters of galaxies. Since 3C449 is located in a cluster of galaxies (albeit a poor one) and since HEAO 1 data[21] demonstrate that poor clusters can also contain X-ray emitting gas, it seems that the radio lobes may be statically confined by external thermal pressure. The diffuse trailing emission is then interpreted as gas rising out of the intracluster medium through buoyancy forces, the overall symmetry of the large scale features being due to the central location of 3C449 in the cluster.

Table 3 shows that in both the northern and southern jets the quantity BR is approximately constant, where B is the magnetic field strength and R is the path length through the jet. This result implies that $B \propto r^{-1}$, where r is the cross-sectional radius of the jet, as suggested by Blandford and Rees[8] for a jet with a circumferential field. A similar result was found by Readhead *et al.*[2] for the jet in NGC6251, and it would be interesting to measure the orientation of the magnetic field in that source.

We now consider depolarisation within the jets. They have a typical degree of polarisation of ~30% and, since the electric vectors are quite uniform, it seems that beam depolarisation is unlikely at 6 cm. Thus we must inquire whether the observed degree of polarisation indicates the intrinsic degree of ordering of the magnetic field or whether significant depolarisation due to thermal matter in the jet has already occurred at 6 cm. We first consider the latter possibility and note that Westerbork 21-cm observations of the jet in NGC315 (ref. 5) show that jet to have an intrinsic degree of polarisation of up to 60%. Adopting this value as a possible intrinsic polarisation for 3C449 and using the simple slab depolarisation model of Burn[22] we find a rotation measure internal to the jet of $\sim|500|$ rad m^{-2}. If the path length is ~3.4 kpc then $nH_z \sim 1.8\times10^{-7}$ cm^{-3} G, where n is the thermal matter density and H_z is the line of sight component of the magnetic field. $H_z \lesssim \sim 10^{-5}$ G (Table 3) so $n \gtrsim \sim 0.02$ cm^{-3}. An internal rotation measure of $\sim|500|$ rad m^{-2} would imply that the average degree of polarisation in the jet at 21-cm wavelength is only ~4% if the slab model means anything. The unpublished 21-cm Westerbork observations of Bystedt and Högbom show the jet polarisation to be ~4%, a value in agreement with what we find.

The low 20-cm polarisation may be due to differential galactic Faraday rotation across the 20-cm WSRT beam. This would require a gradient in the rotation measure of ~50 rad m^{-2} over an angular scale of 35″. This is an extremely large gradient, and although no information on rotation measure gradients over this angular scale is available, we regard this explanation for the low 20-cm polarisation as unlikely. The issue will only be settled by high resolution 20-cm polarisation measurements of the jet—planned for late 1979.

We consider now the possibility that the 6-cm 30% polarisation of the jets represents the intrinsic degree of polarisation. In that case a considerable amount of energy is stored in a random magnetic field. Since the 21-cm degree of polarisation is ~4% (ref. 14) there is strong depolarisation by 21 cm. In fact, 21-cm polarisation divided by intrinsic polarisation equals 0.13 and, with this large depolarisation, the rotation measure could be any of several values as we could have gone through the first minimum of the $\sin\chi/\chi$ depolarisation curve appropriate for a simple slab. However, the minimum acceptable rotation measure is $\sim|75|$ rad m^{-2}, which implies $nH_z \gtrsim 3.4\times10^{-8}$ G cm^{-3}. The uniform component of magnetic field is, in this case, $\lesssim \sim 5\times10^{-6}$ G so $n \gtrsim \sim 0.004$ cm^{-3}. This limit for the thermal density is not greatly different from that found for the previous case, and since $|75|$ rad m^{-2} is a minimum rotation measure, we adopt an internal thermal density of 0.02 cm^{-3}. This value is in good agreement with the density derived for the jet in 3C31 from dynamical arguments[23]. Since the 3C449 jets seem to have a large internal rotation measure, the integrated rotation measure[18] may not affect the direction of the jets and our derived magnetic field direction cannot yet be considered definitive.

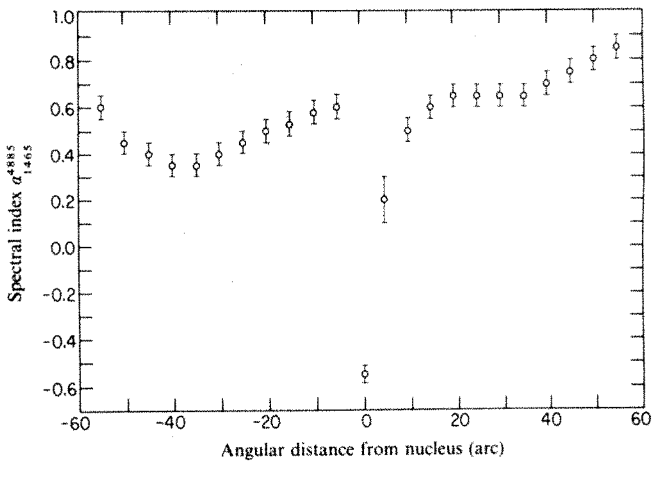

Fig. 4 The measured spectral index of the jets, defined through $S_\nu \propto \nu^{-\alpha}$. The error bars are estimated from the noise in the data, and do not include the possible effects of unmeasured brightness components. The effect of such components will be to lower generally the measured values of α.

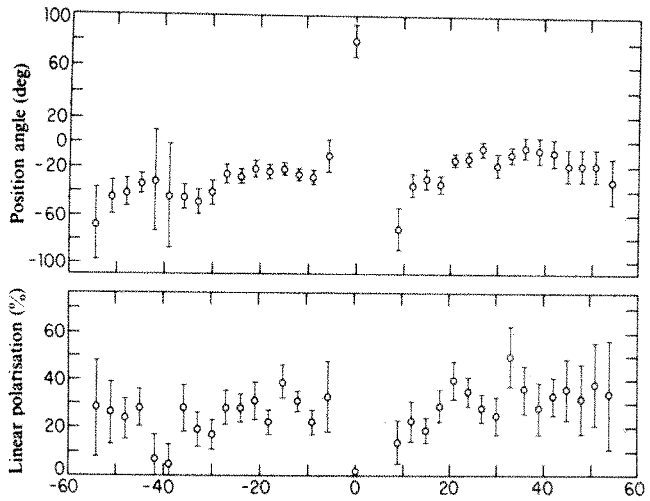

Fig. 5 The percentage and position angle of linear polarisation in the centre of the jets in 3C449 at 6 cm. In this case, the errors have been calculated using the law of propagation of errors from the observed errors in the Stokes parameters *I*, *R*, and *U*. The systematic error due to the polarised side lobes of the VLA antennas has not been included; however, these errors are negligible as they are <1% within the angular extent of the jets.

At distances greater than ~10 arc s from the nucleus the jets expand with a uniform opening angle of ~7°. If the jet is free (not confined by a surrounding medium), the transverse expansion will be at approximately the generalised sound speed, $(\mu/\rho)^{1/2}$, where μ is the internal energy density and ρ is the mass density. A lower limit to the internal energy density in the jet is given by the energy density in relativistic particles and fields, $\sim 7\times 10^{-1}$ erg cm^{-3} (Table 3) while we estimated ρ to be $\sim 3.3\times 10^{-26}$ g cm^{-3} from the depolarisation calculations. The resulting sound speed is ~150 km s^{-1}. From the opening angle, we find a Mach number of 8(= cotan 7°) which implies a flow speed of ~1,200 km s^{-1}. This velocity compares favourably with the flow velocity in the jet of 3C31 derived on the basis of dynamical arguments[23]. Furthermore, this value is comparable to that suggested[24] for the velocity at which material is ejected from the nucleus of the head-tail galaxy NGC1265.

Our estimate of the flow speed depends on the assumption that the transverse expansion is at the internal sound speed, C_s. We note that in an expanding jet, C_s will decrease with increasing distance from the nucleus as the flow becomes more ordered. Thus the lateral expansion must be at least C_s, and so our derived flow speed is a minimum. Furthermore, if the jet is thermally confined, the flow speed will be higher than for a free jet of the same width.

The decline of the internal sound speed with distance from the nucleus may be expected to cause a decrease in the jet opening angle with distance. Note that such a curvature may be occurring in the inner 10″ of each jet, as seen in Fig. 3.

Further evidence for flow speeds of the order we have estimated is given by the changes in the spectral indices along the jets (Fig. 4). The steepening of the spectrum seen at distances greater than ~35 arc s from the nucleus indicates (from synchrotron ageing arguments)[20] that the age of the jet at this point is $\sim 1.1\times 10^{7}$ yr. This distance then translates into a velocity of 800 km s^{-1}. Considering the uncertainties in these types of calculations, we feel that this value is in good agreement with our previous estimates.

The flow speed of ~1,000 km s^{-1}, combined with a mass density of 3.3×10^{-26} g cm^{-3} gives a mass flow of 2×10^{26} g s^{-1}, corresponding to 3 $M_\odot$ yr^{-1}. This mass flow corresponds to an energy flow of 1.5×10^{42} erg s^{-1}, a quantity which exceeds the directly visible energy flow in relativistic material by a factor of ~20, and which may be a significant source of energy available for production of relativistic matter in the outer lobes.

We have previously noted that each jet bends towards the east before terminating in large, diffuse lobes, and that these parallel bends rule out rotation of the nuclear source. It may seem attractive to invoke the radio-trail hypothesis[25] to explain these bends, but because the lobes bend back towards the axis defined by the jets, this hypothesis seems unlikely. Rather, the similarity of the structure of 3C449 to that of 3C31 (ref. 6) suggests that the gravitational encounter hypothesis of Blandford and Icke[23] would be more successful in explaining the bends in 3C449. In this hypothesis, Blandford and Icke have had at least partial success in explaining the symmetry of 3C31 by invoking a gravitational interaction between 3C31 and a nearby companion. Since 3C449 has two companions[26] located within 100″, the symmetry in this source may be due to a similar interaction between one or both.

Beyond the large lobes, the emission is seen to trail away with a small but distinct curvature towards the west. This structure is reminiscent of the general structure of radio tail galaxies, and it is worth noting other similarities between 3C449 and radio tail galaxies such as 3C83.1B (NGC1265). The luminosity of 3C449 is typical of radio tail galaxies, and the observation that the compact structure of 3C449 is located close to the optical galaxy is also similar to NGC1265. Also, our derived flow speed (~1,000 km s^{-1}), mass flow rate ($\sim 3M_\odot$ yr^{-1}) and ratio of bulk kinetic to relativistic energy (20 : 1) agree with theoretical values taken from the fit of the plasmoid model[27] of radio tail galaxies.

There is increasing observational evidence that gaps near the nuclear source seem to be a common property in radio jets, having been observed in several radio sources (for example, NGC 1265[28], NGC 315[5] and 3C219[3]). The typical physical separation is usually several kpc. At present we can only suggest some explanations. The gaps may represent the intervals between major outbursts. The typical separation between major peaks in the 3C449 jets is 3.4 kpc, corresponding to $\sim 3\times 10^{6}$ yr with our previously estimated flow speeds. This time interval between major outbursts is in good agreement with that calculated[24] for NGC1265. Another suggestion is that the material flowing from the nucleus is initially very highly collimated so that synchrotron radiation is inhibited by a small pitch angle between the particles and magnetic field. This suggestion implies that close to the nucleus the magnetic field should be parallel to the jet. VLA observations of 3C31 and NGC315[29] suggest that this configuration may indeed prevail. Alternatively the magnetic field could be much lower than the equipartition value (a decrease by a factor of 20 would suffice to produce the observed drop in brightness). If either of the last two suggestions holds true, then some agency, possibly turbulence, is required to randomise the particle pitch angles or amplify the magnetic field strength, or both, by the time the material has travelled several kpc from the nucleus.

This latter suggestion may relate to the changes in spectral index and 6-cm degree of polarisation along the jets (Figs 4 and 5). In the northern jet, the spectrum continually steepens as the distance from the nucleus increases; at the same time, the degree of polarisation steadily increases, which would indicate an increasingly ordered magnetic field. The spectrum of the southern jet flattens over the distance 0–35″ from the nucleus while the degree of polarisation apparently decreases over this

Table 3 Equipartition values of magnetic field and total energy density at selected points in 3C449

Site	Emissivity* I/S (erg s^{-1} cm^{-3} sr^{-1})	$H_{\min}$ (μG)	$\mu_{\min}$ (erg cm^{-3})
Southern Lobe	4.5×10^{-29}	7.3	4.9×10^{-12}
Northern Lobe	3.8×10^{-29}	7.0	4.5×10^{-12}
Southern Jet 40″ S	2.1×10^{-28}	8.1	3.9×10^{-12}
Southern Jet 15″ S	6.8×10^{-28}	11.2	1.2×10^{-11}
Northern Jet 15″ N	5.6×10^{-28}	10.6	1.0×10^{-11}
Northern Jet 35″ N	1.6×10^{-28}	7.4	5.1×10^{-12}

* From 10 MHz to 10 GHz assuming a power law spectrum of index 0.5 for the jets and 0.7 for the lobes.

distance, although the errors are large. Thus the magnetic field in the southern jet apparently becomes increasingly disordered. This disordering might be due to turbulence which then might also be responsible for transferring energy to the relativistic electrons[27] and thus for flattening the observed radio spectrum.

Conclusions

The low-luminosity radio galaxy 3C449 exhibits great variation of structure. Two compact, well collimated, and nearly symmetric jets are found to issue from an unresolved core which is optically thick at 6 cm and coincident with a cDE4 optical galaxy. The radio jets are not smooth in appearance, showing a lumpy and somewhat turbulent structure. The jets are not seen within 10″ (~3 kpc) of the central core, but appear to 'turn-on' at this distance. Each jet widens steadily beyond the turn-on point, and gradually diminishes in brightness until each makes a sharp bend to the west and terminates in large, diffuse blobs with very relaxed appearance. Trailing emission up to ~8′ from the centre is detected.

The spectrum of each jet is flatter than normal, with the spectral index averaging near 0.6. Little trend in the index is seen, although the southern jet may flatten slightly towards the end. Both jets are highly polarised, with percentage linear polarisation varying generally from 20 to 40%. The position angle of the polarisation is nearly constant throughout each jet. If all, or most, of the Faraday rotation is external, then the inferred jet magnetic field is circumferential. However, a significant portion of the rotation measure may be due to internal thermal matter as WSRT observations of the jet show low polarisation. If this depolarisation is due to internal Faraday rotation, the inferred electron density is $\sim 0.02\ \text{cm}^{-3}$. Combining this mass density with minimum energy densities derived from standard equipartition arguments, a minimum flow speed of $\sim 1{,}000\ \text{km s}^{-1}$ for a free jet is found.

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Jupiter's rings

Tobias Owen*, G. Edward Danielson†, Allan F. Cook‡, Candice Hansen§, Virginia L. Hall† & Thomas C. Duxbury§

* Department of Earth and Space Sciences, State University of New York, Stonybrook, L.I., New York 11790
† Division of Geological and Planetary Sciences, California Institute of Technology, Pasadena, California 91125
‡ Smithsonian Astrophysical Observatory, Cambridge, Massachusetts 02138
§ Jet Propulsion Laboratory, Pasadena, California 91103

Voyager 2 observations of the jovian ring system discovered by Voyager 1 are presented. The rings were observed both above and below the jovian equatorial plane and at extremely low and high phase angles. This ring system seems to represent a steady state configuration for small particles that are slowly moving in toward Jupiter. It must be resupplied from sources well outside Roche's limit.

JUPITER'S ring system was discovered on 4 March 1979 by Voyager 1 as a result of a deliberate search as the spacecraft passed through the equatorial plane of the planet[1]. Having established the existence and outer dimension of a jovian ring system, Smith *et al.* worked with the Voyager engineers to develop new imaging sequences for the Voyager 2 encounter with Jupiter during early July. The major goal of these sequences was to make observations slightly out of the ring plane, in order to establish the number of ring components present, their dimensions and relative brightness. Several filters were used to establish the colour of the ring particles. A summary of Voyager's jovian ring data set is given in Table 1. Figure 1 illustrates the geometry and times at which Voyager 2 data were taken with respect to the flyby trajectory.

Data description

The first set of newly sequenced images was obtained on 8 July at an inclination of 2.5° to the ring plane on the inward leg of the trajectory. These data were the first to reveal both sides of the ring as seen in Fig. 2. This view confirmed that the jovian ring was very faint and showed no obvious gaps. The brightest segment of the ring in Fig. 2 was found to be $\sim 3\times 10^{-5}$ of the mean brightness of Jupiter at zero degrees phase angle. These data were smeared by relative spacecraft motion (apparent in the star trails recorded in 96-s exposures).

The next set of images was obtained 14.5 h later as the spacecraft passed through the planet's equatorial plane. Because of image motion, these pictures did not immediately add to the information already available from Voyager 1, but they confirmed the existence of a sharp outer edge for the ring system (Fig. 3). This boundary has been remeasured on the Voyager 1 image and has a value 1.8 R_J (128,500 km from the centre of Jupiter).

All of these observations (including the Voyager 1 picture) were obtained at rather small phase angles; the rings were visible because of light scattered back towards the camera. At the time of the Voyager 2 encounter the Sun was in Jupiter's equatorial plane with about two thirds of the solar disk below (south of) the ring plane as seen from Jupiter.

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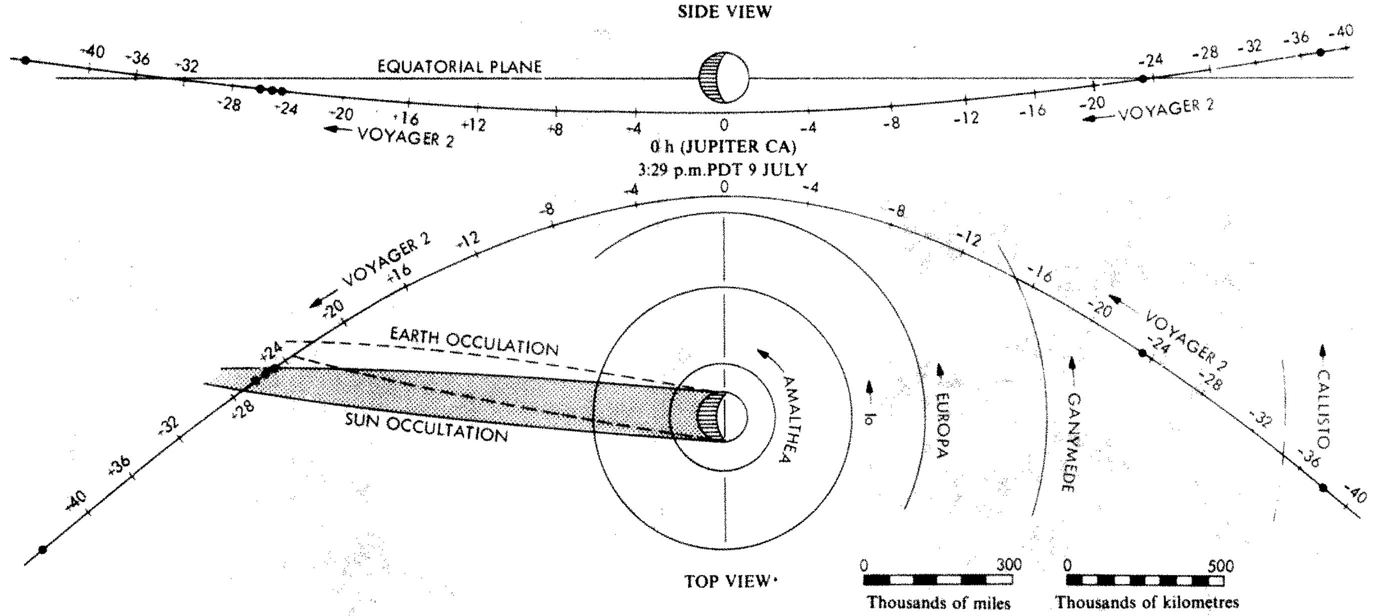

Fig. 1 The Voyager 2 spacecraft's Jupiter flyby trajectory is shown in two views. Each of the sets of pictures targeted for the rings is shown by the symbol ●. The time of closest approach for Jupiter was 11.21 UT 9 July 1979 (4.21 p.m. PDT 9 July). The range at closest approach was 10 R_J.

Twenty-six hours after closest approach to Jupiter, the spacecraft was in Jupiter's shadow approximately two degrees below the ring plane. The pictures taken in these illumination conditions at very high phase angles, resulted in the spectacular images shown in Figs 3, 4, 5, 6 and 7. Close inspection of the complete set of pictures indicates that there are at least four distinct components in the jovian ring system:

(a) a narrow bright segment about 800 ± 100 km wide, inner edge 1.7 R_J;

(b) a fainter segment about 6,000 km wide, inner edge 1.68 R_J;

(c) A very faint inner 'ring' that appears to be a continuous sheet of dispersed material probably extending all the way to the planet;

(d) an even fainter outer edge of small particles that extend to 1.8 R_J and slightly beyond.

Each of these components is designated in the intensity plot of Fig. 8. The intensity plot was taken normal to both sides of the ring halfway between the visible end of the ring and Jupiter.

Note that the jovian ring system is thus closer to its parent planet than either of the rings of Saturn (outer edge of ring A = 2.28 R_S) or the rings of Uranus (outer edge of ring ε = 2.1 R_U). On the other hand, the width of the jovian ring is intermediate between the extremely narrow rings of Uranus (<100 km) (ref. 2) and the broader rings of Saturn (17,000–25,000 km).

Steady-state rings

We favour the following hypothesis for the origin of the rings and their maintenance: namely, that the rings seem to represent a steady-state configuration for small particles moving in towards the planet in orbits near the equatorial plane. The particles are supplied from sources outside the Roche limit which could include cometary and meteoritic debris, impact ejecta from the inner satellites, and volcanic ejecta from Io. Evidence of loss from the ring is apparent from the observed distribution of particles inside the bright segment, ring (c). Among the loss mechanisms to be considered we note the Poynting–Robertson effect both from direct sunlight and that reflected by Jupiter, but interactions with the jovian magnetic field and the environment of charged particles are probably more important. Braking by the jovian magnetic field (which is moving more slowly than particles in the ring) will cause charged grains to move inwards. The high energy ions in the jovian magnetosphere will sputter material from them, causing the particles to get smaller and thus enhance the previously mentioned braking mechanisms.

Preliminary analysis

A rough estimate of the mean particle size in the jovian rings can be obtained from the phase function manifested in the strong forward scattering. In the narrow angle frames, it is evident that the arm of the rings closer to the spacecraft is less bright than the farther one. This is shown in Fig. 8 where a plot of relative intensity is shown for both the near and far sides. This set of data represents a difference in scattering angle of ~0.3°. Inspection of intensity contours for single particle scattering[3] suggests that for this condition to exist, the size parameter $X = 2\pi a/\lambda$ must be about 50. Adopting $\lambda = 0.497$ μm as the effective wavelength of the narrow angle camera with the clear filter (a clear quartz substrate) we find that the mean particle radius is in the range of 4 μm. This estimate is also supported by comparing the brightness of the scattering at this high phase angle with that obtained near opposition. The Voyager 2 data show that the rings are approximately 20 times brighter in the diffraction lobe than in backscattering. (Note: the actual maximum value of this difference must be greater still.)

It is also evident from colorimetry based on comparisons of images taken through violet, orange and green filters that the light diffracted towards the spacecraft by the ring particles at phase angles near 180° is distinctly reddish compared with sunlight (the ratio of orange to violet is 1.7–1). This suggests either blue absorption by the particles or wavelength-dependent forward scattering (diffraction). At this stage, it is not possible to determine which of the two causes is dominant. Note that low

Table 1 Voyager 1 and 2 observations of jovian ring system

Date/time UT	No. of pictures	Filter	Camera*	Exposure time (s)	Angle†
4 March/19.38	1	Clear	NA	672	0°
8 July/8.39	2	Clear	NA	96	+2.5°
8 July/23.04	6	Clear	NA	96	0°
	1	Violet	NA	96	
	1	Green	NA	96	
	1	Orange	NA	96	
	1	Clear	WA	15	
10 July/00.48	2	Orange	WA	96	−2.0°
	2	Violet	WA	96	
	7	Clear	NA	96	
	1	Clear	WA	15	

* NA, narrow angle; WA, wide angle.

† The angle of the spacecraft above or below the jovian ring plane.

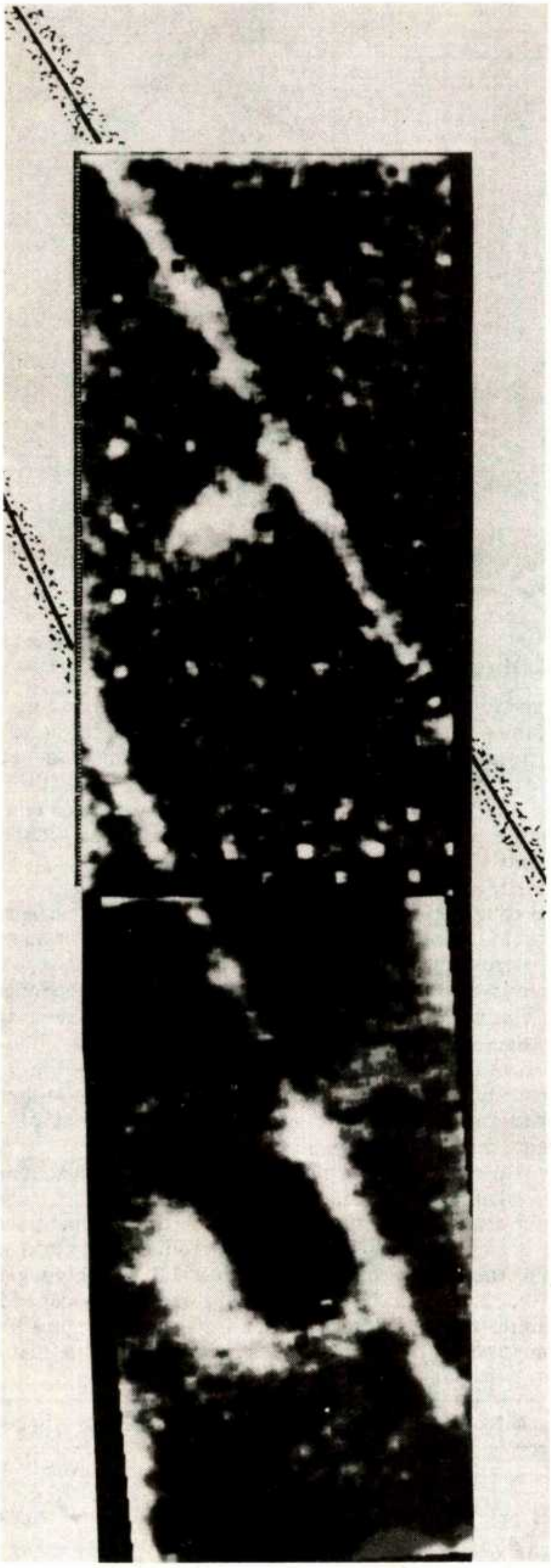

Fig. 2 Both sides of the jovian rings are apparent in this two picture mosaic returned by Voyager 2. These data were taken at a range of 2×10^6 km when the spacecraft was approximately 2.5° above the ring plane. The data were submitted to an extreme contrast enhancement to bring out this faint signal. The blotchy areas around and between the rings are artefacts of this extreme enhancement.

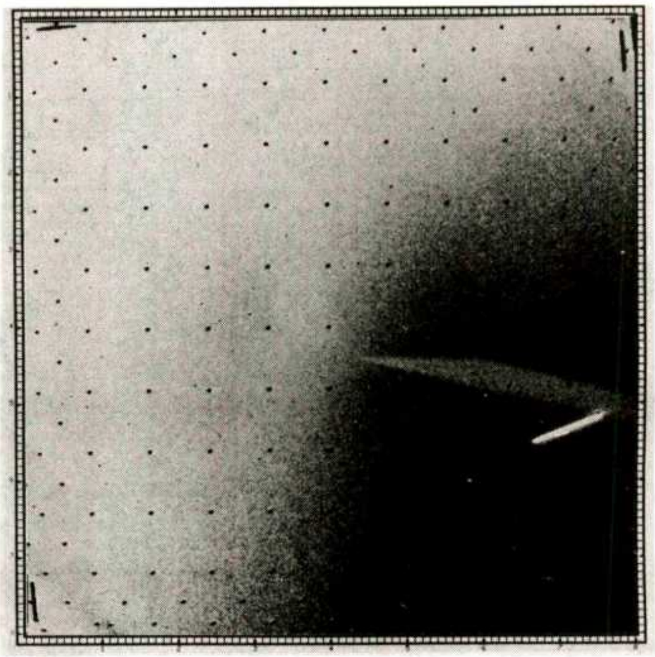

Fig. 3 This Voyager 2 picture caught the sharp outer edge of the ring as the spacecraft passed through the ring plane at a range of 1.4×10^6 km from Jupiter. The bright star trail shows the relative spacecraft motion which smeared the picture in this 96-s exposure through the clear filter. This was one of a set of nine pictures targeted at this time.

reflectivity of blue light is consistent with the low blue albedos of all five large jovian satellites (especially JV and JI) and most asteroids and meteorites[1,4,5]. The colour by itself, however, is not diagnostic of composition.

Resonance commensurabilities

The existence of a well-defined outer boundary for the rings suggests a decrease in the drag force acting on inward evolving particles moving into this general region. The fact that the brightest segment of the ring (a) is at this outer boundary simply reinforces this impression. There are two near commensurabilities between the period of particles at the ring's outer edge and fractional periods of jovian satellites:

$$\tfrac{3}{5}p_{\text{Amalthea}} \text{ and } \tfrac{1}{6}P_{\text{Io}} \text{ (in days: } P_{(\text{a})}=0.291,\ \tfrac{3}{5}P_{\text{v}}=0.299,\ \tfrac{1}{6}P_{\text{I}}=0.295)$$

These near commensurabilities do not seem particularly compelling, as the mass of Amalthea is very small ($\sim10^{21}$ g if $\rho=3.4$) and 1/6 is a small fraction of Io's period. For comparison, the outer edge of Saturn's A ring falls close to a 2/3 resonance with Mimas, while particles in Cassini's Division would have approximately half the period of Mimas, but even there the agreement is not perfect[6]. Note that if Amalthea were considered to have a role equivalent to Mimas for the Jupiter system, the entire (a)+(b) complex falls well inside the boundaries set by the 1/2 and 2/3 resonances. It may be that in the Jupiter system the satellite resonances only serve to modulate other effects, for example, a balance between electrostatic and gravitational forces.

Relation to Roche limit

In any case, the rings are well inside the classical Roche limit for the breakup of a liquid satellite (2.44 R_{J}) and also within the limit set by the mutual gravitational attraction between two particles with mean densities differing from that of Jupiter:

$$r = R_{\text{J}}\left(16\frac{\rho_{\text{J}}}{\rho_{\text{P}}}\right)^{1/3}$$

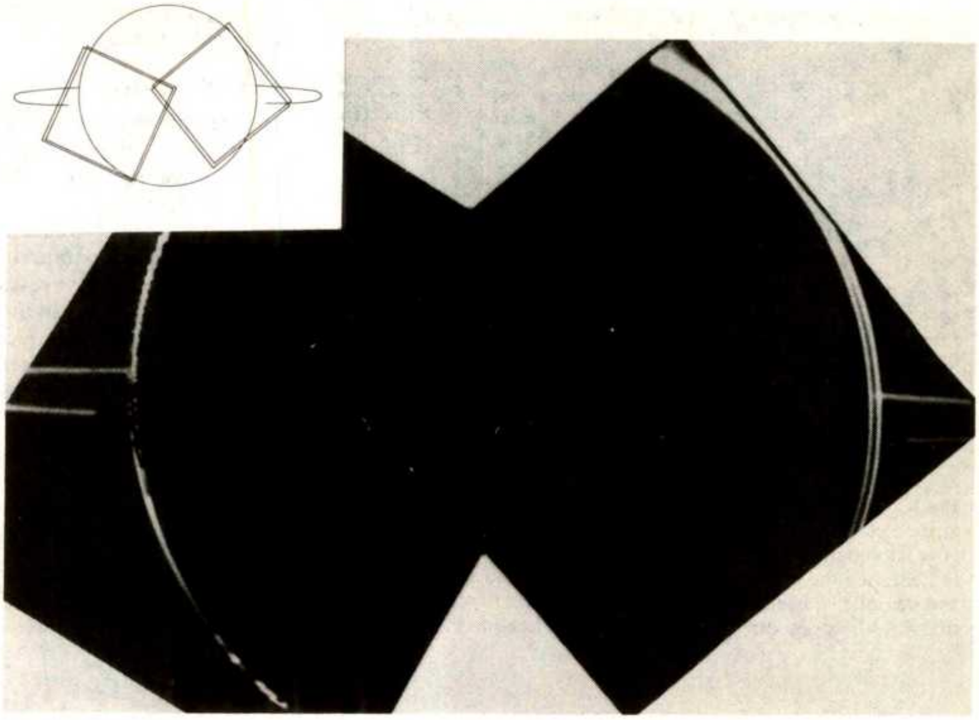

Fig. 4 This mosaic was taken while the spacecraft was in Jupiter's shadow at a range of 1.45×10^6 km. These pictures were taken with Voyager 2 spacecraft's wide angle cameras through the violet filter. The limb of Jupiter and the rings are clearly revealed in these high phase angle pictures as a result of forward scattering from a haze layer high in Jupiter's atmosphere.

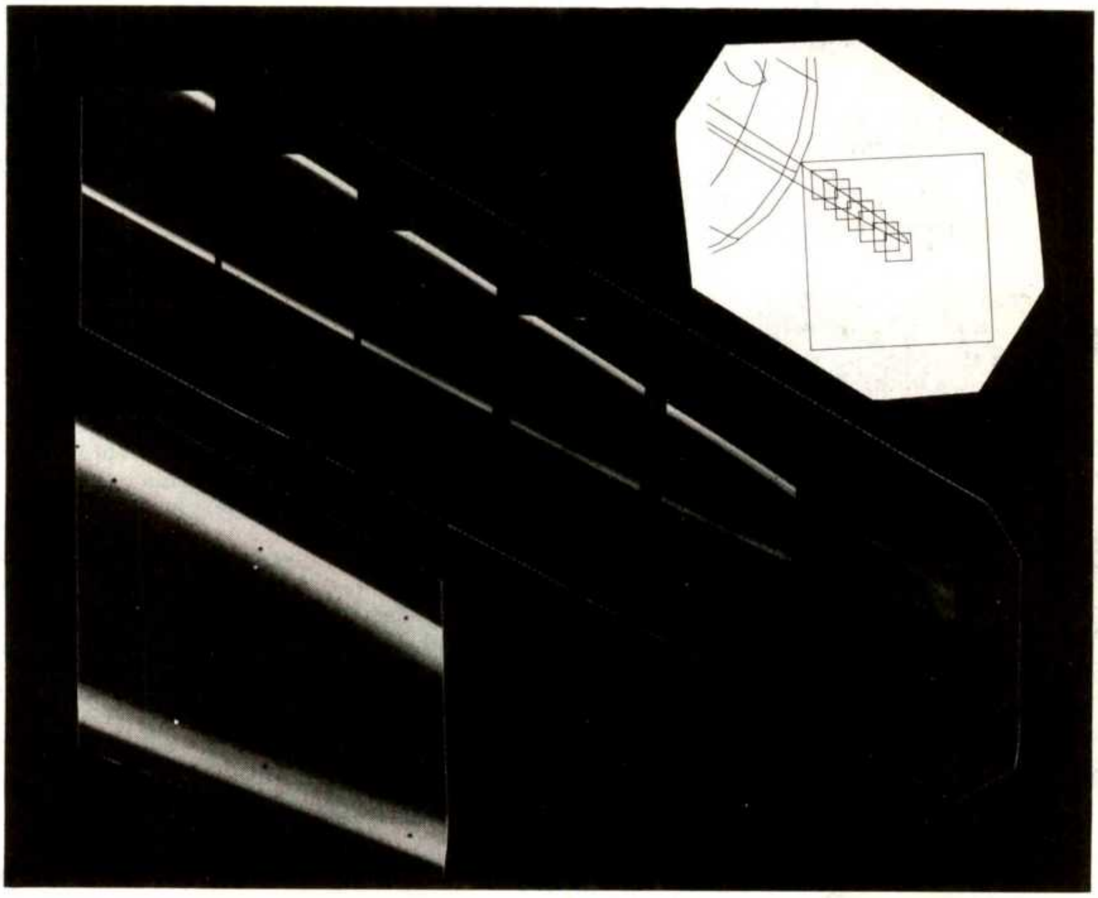

Fig. 5 This six picture narrow angle sequence was taken at high phase angles while the Voyager 2 spacecraft was in Jupiter's shadow. The line drawing in the upper corner illustrates the targeted fields of view predicted for this picture-taking sequence. These data were edited onboard the spacecraft to preserve the signal-to-noise ratio required to send them back in real time (which accounts for their rectangular shape and the loss of overlap). The picture on the lower left has been enlarged to illustrate the presence of material between the bright arms which forms a continuous sheet that may extend all the way to the planet. The star trail shown gives a history of the smear during this 96-s exposure.

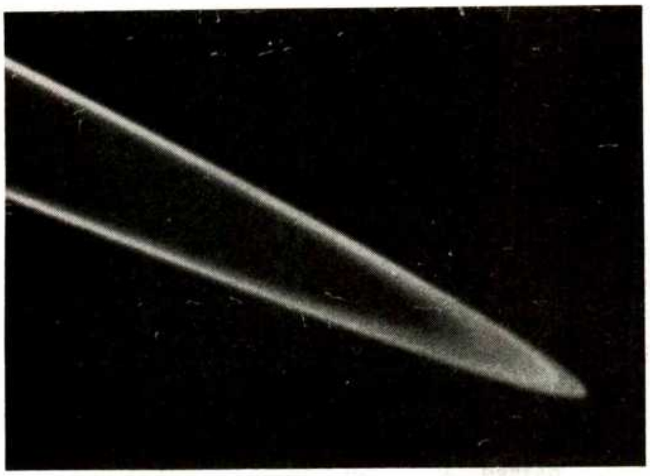

Fig. 6 This wide angle picture was taken at the end of the narrow angle picture sequence in Fig. 5. It shows the bright, sharp outer edge, along with the fainter inner continuous sheet of particles (ring (c)) between the edges. This picture had an exposure time of 15 s and was taken through the clear filter. The spacecraft was at a range of 1.4×10^6 km in Jupiter's shadow about 2° below the plane of the ring.

Adopting $\rho_p\approx2.6$ (for example, meteoritic material) $r=2\ R_J$. On the other hand, if Amalthea represents a high temperature condensate that continues the density gradient from JIV to JI, it might have a density as high as 3.5, the mean density of Io. Fragments of this satellite in the ring would then lead to $r=1.81\ R_J$, close to the observed outer radius. As the particles in Saturn's rings evidently consist mainly of water ice, this gravitational constraint suggests that one reason Jupiter's rings are so close to the planet may be that the ring particles have a higher mean density than the particles in the rings of Saturn.

The limit for the breakup of a solid body with a diameter larger than 30 km and a very low tensile strength (10^6 dyn cm^{-2}) has been calculated by Aggarwal and Oberbeck[7] to occur between 1.35 and 1.38 R, well inside the observed dimensions of the (a) and (a)+(b) components of the ring ($1.68-1.77\ R_J$). Hence, there is no reason to assume we are looking at fragments from a disrupted satellite.

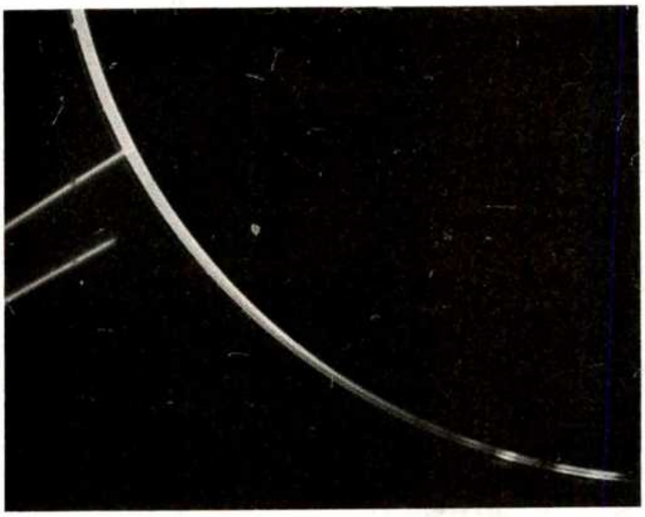

Fig. 7 A black and white reproduction of a colour composite which was made from a violet and orange frame taken with the wide angle camera. These are the same data as shown on the left in Fig. 4. The jovian ring system is two light orange lines protruding from the left along with the very faint orange materials in between them. The edge of the ring closest to the spacecraft has been cut off by Jupiter's shadow. The spacecraft was approximately two degrees below the ring plane. The very colourful multiple images of the Jupiter limb are evidence of image smear during these long exposures (96 s).

Conclusions

Before this discovery, Pollack[8] suggested that a ring around Jupiter was unlikely because original particles left over from the planet's accretion would have been quickly lost by gas drag and ice particles would not have condensed late in the accretion ring dissipation stage because Jupiter would have been too hot. Our model for a steady-state ring is entirely consistent with this point of view. It is now necessary to define the sources, sinks and stabilising influences in more detail.

Further study of the pictures of Jupiter's ring should give an improved assessment of the scattering properties and hence average size of the ring particles. The dimensions for the ring components stated here will be refined and the degree to which the ring approaches a perfect circle will be determined with the help of the images of stars recorded on individual frames. Unfortunately, we will not be able to improve the crude upper limit of 30 km placed on the ring thickness by Voyager 1, as the images obtained at ring-plane crossing with Voyager 2 do not give any better resolution.

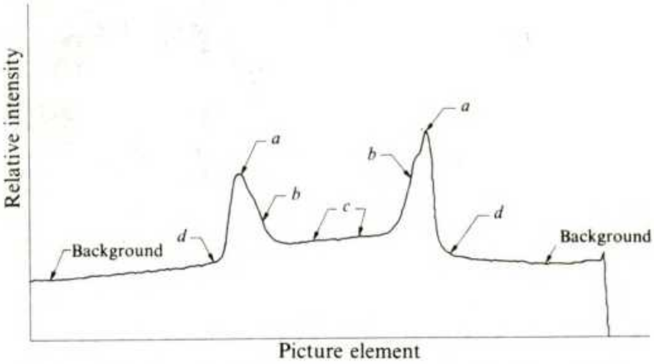

Fig. 8 This plot of relative intensity illustrates the difference in brightness and resolution between the near and far edge of the rings. These data were taken perpendicular to the ring at about 1.4 R_J. This narrow angle frame is part of the mosaic in Fig. 5 taken through the clear filter. It shows a ratio of ~1.43 for the brightest part of each side of the ring plotted in this intensity profile. This plot also clearly shows each of the four components of the jovian ring system. The brightest segment (*a*) and the fainter and wider inner ring (*b*) along with the very faint components (*c*) and (*d*).

We thank the many JPL and NASA personnel who worked on Voyager and made the successful flybys of Jupiter possible. We also thank Dr J. E. Hansen, L. Moriboto and S. Synnott and the navigation team for their support in data reduction calculations. This paper represents the results of one phase of research carried out at the JPL and NASA contract NAS 7-100.

Note added in proof: We have searched carefully for evidence of a shadow from the rings on the surface of the planet, using Voyager 1 images when lighting conditions were optimal. No indication of a shadow was found, even though our resolution was twice the expected shadow width (~50 km). Pre-discovery reports of ground-based observations of the shadow—at resolutions more than 150 times worse than that of the spacecraft—are probably incorrect.

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Directive segregation is the basis of ColE1 plasmid incompatibility

John R. Bedbrook*, Hans Lehrach† & Frederick M. Ausubel

Department of Biology, Biological Laboratories, Harvard University

Incompatibility between ColE1 plasmids in Escherichia coli *can be explained by competition for a limited number of replication sites. These sites ensure directive segregation of plasmids to daughter cells on cell division. The number of sites can be more than one only if a linear segregation mechanism is postulated.*

Plasmid incompatibility is defined as the failure of two plasmid replicons to be stably maintained in the same bacterial cell. Two general types of models have been postulated to explain plasmid incompatibility. One is derived from the replicon hypothesis of Jacob, Brenner and Cuzin[1]. They proposed that each bacterium has a single site at which particular plasmids are replicated. The site acts as a directive segregation mechanism which ensures that each daughter cell receives a copy of the attached plasmid. Incompatibility is due to competition between plasmids belonging to the same compatibility group for this single essential replication site. A second model, proposed by Pritchard and coworkers[2-3], is based on the observation that an Hfr strain cannot harbour a free F factor. According to this model, incompatibility results indirectly from the action of trans-acting cytoplasmic factors (repressors) whose actual physiological function is to regulate plasmid copy number. When two plasmids produce and are sensitive to the same repressor, there is an increased probability that a daughter cell will not receive both plasmids because the combined copy number of the two plasmids is equal to the normal copy number of each plasmid alone.

Plasmid incompatibility among small non-conjugative plasmids has been investigated[4,5], and Warren and Sherratt[6] have recently demonstrated that any two plasmids derived from ColE1[7] replicons are incompatible. They found that smaller ColE1 derivatives displaced larger ones and argued that this was consistent with a replication repressor model for plasmid incompatibility. The conclusion was based on the assumption that smaller plasmids have less affinity for the postulated replication repressor than larger plasmids. There is now evidence for the existence of replication repressors[8]. These experiments indicated that pSC101[9] copy number is controlled by a trans-acting factor.

In the experiments reported here, we have used two families of multicopy plasmids to investigate further the mechanism of plasmid incompatibility. One family is characterised by ColE1 and the other by RSF1030[10]. Our results can be summarised as follows: (1) RSF1030 and its derivatives were compatible with ColE1 and its derivatives; (2) when two ColE1 derivatives were used to simultaneously transform *rec*A$^-$ *E. coli* resultant double transformants existed only temporarily; (3) the hybrid RSF1030-ColE1 replicon and a single ColE1 replicon were stably maintained in the same cell; and (4) multimer forms of pMB9[11] were incompatible with each other. Our results support the view that plasmid incompatibility is the consequence of a directive segregation mechanism for plasmid propagation. The phenomenon of incompatibility is not associated with plasmid copy number control and the results are not consistent with a model postulating that replication repressors are the primary cause of plasmid incompatibility.

Co-transformation of *E. coli* by two plasmids as a system for plasmid incompatibility studies

One way to study plasmid incompatibility is to simultaneously introduce a single copy of each of two phenotypically distinguishable, incompatible plasmids into a single *E. coli* cell. Figure 1 demonstrates that significant numbers of double transformants (arising by simultaneous transformation with two incompatible ColE1 plasmids) could be obtained at DNA concentrations at which double transformants were the result of two independent single-hit events by the two incompatible plasmids. The number of cells obtained that were resistant to a single drug was directly proportional to the concentration of DNA used in the transformation and the saturating DNA concentration of 6×10^{-9} M was the same for each plasmid. We have no simple explanation for the observation that the number of transformants obtained at any particular molar concentration of DNA was greater for RSF1030 than for the ColE1 derivatives pML21[12] and pMB9 and in turn was greater for pMB9 and pML21 than for another ColE1 derivative pSF2124[13].

Formation of double transformants with two compatible plasmids (RSF1030 and pML21 or RSF1030 and pMB9) was also log-linearly related to plasmid DNA concentration. The slope of the line log(number of transformants) versus log(DNA concentration) is ~2, as expected for two independent random events. The number of double transformants also plateaued at 6×10^{-9} M DNA. The formation of pMB9–pML21, pMB9–pSF2124 or pML21–pSF2124 double transformants were not log-linear with DNA concentrations. Nevertheless, significant numbers of double transformants were obtained within the range of plasmid DNA concentrations at which double transformants for compatible plasmids arose by two independent events. We have ruled out the possibility that double transformant colonies are a consequence of mutual crossfeeding of plasmid products from separate pMB9 and pML21 transformants. When pML21-containing cells and pMB9-containing cells were patched together at high concentrations on a plate containing tetracycline (Tc) and kanamycin (Km) no growth of the patch was observed.

Genetic instability and plasmid composition of cells of double transformant colonies

A *rec*A$^-$ strain (HB101) was simultaneously transformed with pMB9 (Tc^r) and pML21 (Km^r) and the cells were plated immediately on Tc+Km media. The colonies that grew on the double selection plates were purified on nonselective medium so

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* Present address: Plant Breeding Institute, Trumpington, Cambridge, UK.

† Present address: European Molecular Biology Laboratory, Heidelberg, FRG.

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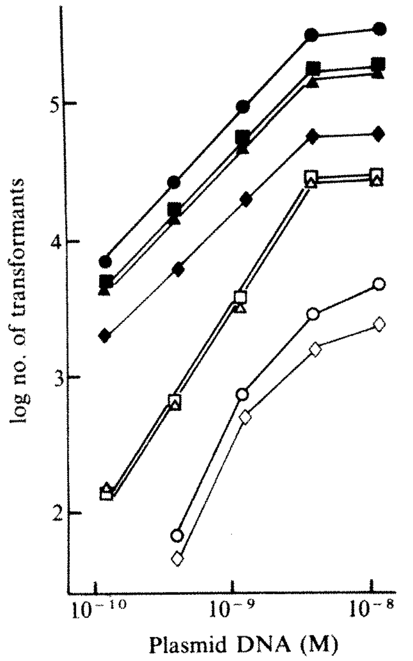

Fig. 1 Frequency of the formation of single and double transformants as a function of plasmid DNA concentration. HB101 ($r_B^- m_B^-$ F^- *pro*$^-$ *gal*$^-$ *arg*$^-$ *rec*A$^-$) (ref. 16) was grown at 37 °C in LB media containing per litre 10 g bacto tryptone (Difco), 5 g yeast extract (Difco) and 5 g CaCl *p*H 7.2. Cells at mid log growth were collected and resuspended in one half growth volume of chilled 30 mM $CaCl_2$, incubated at 4 °C for 20 min, pelleted and resuspended in $\frac{1}{50}$ of the growth volume of 30 mM $CaCl_2$. Approximately 4×10^7 viable $CaCl_2$ treated cells were incubated at 4 °C with mixtures of pMB9, pML21 and RSF1030 plasmid DNAs or pMB9, pML21 and pSF2124 plasmid DNAs at the indicated DNA concentrations for 60 min. The DNA concentration given is the concentration of each plasmid DNA alone. The cell–plasmid DNA mixtures were incubated at 42 °C for 2 min., diluted 10-fold in LB media, and grown for 20 min. at 37 °C. This time was adequate to give the maximum number of transformants. Aliquots of cells at various dilutions were plated on LB media containing 1.5% Difco agar. To select for the presence of RSF1030 and pSF2124, plates contained 30 μg ml^{-1} ampicillin; to select pMB9, plates contained 10 μg ml^{-1} tetracycline; and to select pML21, plates contained 20 μg ml^{-1} kanamycin. RSF1030 (●), pMB9 (■), pML21 (▲), pSF2124 (◆), RSF1030 + pMB9 (□), RSF1030 + pML21 (△), pMB9 + pML21 (○), and pMB9 + pSF2124 (◇). The number of transformants plotted is corrected for a constant viable cell count.

that individual cells from the original colony could be tested for Tc^r, Km^r and Tc^r + Km^r. Each original Tc^{r}Km^r colony contained cells which were either Tc^r or Km^r. No cells were resistant to both Tc and Km. These results indicated that while primary doubly transformed cells must have contained both plasmids, in further generations individual cells propagated only one of the two plasmids.

Figure 2 shows that the proportion of Tc^r cells in a doubly transformed colony that were Tc^r (contained pMB9) varied between 0 and 100% depending on the Tc concentration. (The Km concentration was 20 μg ml^{-1} in all plates and the Tc concentration was 1–10 μg ml^{-1}). We interpret these results as follows: Primary transformants containing both pMB9 and pML21 synthesise products which confer Tc^r and Km^r, respectively. These products are distributed to daughter cells. Some daughter cells propagate pMB9 and others propagate pML21. The cells propagating pMB9 are Tc^r and, for some period of time, are also Km^r, as they received Km^r-conferring products from the primary transformed cell. Similarly, cells propagating pML21 are Km^r and retain a Tc^r phenotype until the Tc^r-conferring products are diluted out. The number of divisions before the critical dilution occurs when daughter cells become Tc^s will depend on the Tc concentration in the medium. Thus the higher the concentration of Tc, the lower the proportion of cells in the double transformant colony that contain pML21. In contrast, at very low Tc concentrations, cells propagating pML21 will have a significant growth advantage over those propagating pMB9 as the latter cells must detoxify the Km in the medium with pML21 products which are continually being diluted at each cell division. Thus, this experiment supports the notion that doubly resistant transformant colonies are a mixture of two cell types, each type propagating one of the two plasmids initially entering the primary transformant.

Table 1 Description of plasmids used

Plasmid	Compatibility group	Description	Relevant phenotype	Source or reference
pMB9	ColE1	pMB1 derivative	Tc^r	11
pML21	ColE1	ColE1 derivative	Km^r	12
pSF2124	ColE1	ColE1 derivative	Ap^r	13
RSF1030	RSF1030	Naturally occurring	Ap^r	10
pJC307	ColE1	Temperature sensitive mutant	will not replicate at 42 °C	15
pHM15	ColE1	pJC307::Tn5::Tn10	Tc^{r}Km^r	H. Meade
pJB28	ColE1	pJC307 + Km^r from pML21	Km^r	This paper
pJB56	RSF1030	RSF1030 + Km^r from pML21	Ap^{r}Km^r	This paper
pJB55	RSF1030 +ColE1	RSF1030-pML21	Ap^{r}Km^r	This paper

Primary double transformant cells replicate both plasmids

The ability of plasmids, temperature-sensitive for replication, to give rise to transformant colonies at the non-permissive temperature for plasmid replication was tested: pJB28, a ColE1 derivative containing the same genes for Km^r as pML21, and pHM15, a ColE1 derivative containing the Tc^r gene (see Table 1), were used to transform the strain HB101. The transformed cells were plated directly at 32 °C and 42 °C over a range of drug concentrations. At drug concentrations adequate to select Tc^r or Km^r, no transformants were obtained at the non-permissive temperature (42 °C), in spite of the fact that the plasmids were entering the cells (as witnessed by the growth of many transformants at 32 °C). Thus, a single copy of a plasmid was not sufficient to allow the growth of a visible colony.

Figure 3 shows that replication of both plasmids must occur in the primary transformed cell. When pJB28 transformants were preincubated at 32 °C before plating at 42 °C, the number of Km^r colonies which formed depended on the length of the preincubation time at 32 °C. The maximum number of colonies was obtained after a preincubation period of 180 min. On retesting, however, less than 0.1% of the cells in colonies forming at 42 °C were Km^r. This experiment showed that a plasmid-containing cell will form a colony on media that selects for a plasmid-coded drug resistance only if plasmid replication is allowed to occur in the cell before plating. Another observation, supporting the view that double transformant colonies arise as a consequence of a period of replication of both plasmids in the primary double transformant, is that the double transformant colony size is inversely related to the length of time that the cell population is grown unselectively before selective plating. This indicates that the number of viable daughters which the plated cell produces depends on how close in the cell lineage it is to the primary transformant.

A site model for plasmid incompatibility

The results described above have led us to the conclusion that when pMB9 and pML21 are used to simultaneously transform *E. coli*, both plasmids replicate in the primary transformed cell.

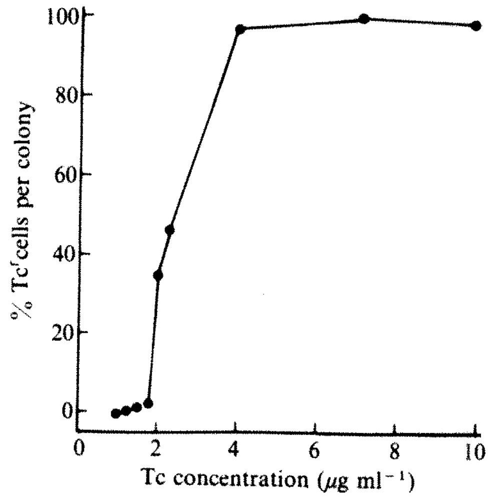

Fig. 2 Plasmid composition of daughter cells of double transformants. pMB9–pML21 plasmid double transformants of HB101 were prepared as described in Fig. 1 legend. Doubly transformed cells were selected on LB agar plates containing Km (20 μg ml^{-1}) and Tc (1–10 μg ml^{-1}). Colonies appearing on double selection plates were streaked onto LB agar plates and grown at 37 °C for 20 h. For each datum point, a minimum of 100 colonies were tested for resistance to Tc and Km.

In subsequent daughter cells only pMB9 or pML21 is able to replicate. The replicon hypothesis of Jacob *et al.*[1] can explain these results. Specifically, we propose that *E. coli* contains a site or sites at which ColE1 plasmids are replicated and that this is the basis of multicopy plasmid incompatibility. Once in occupancy, the resident plasmid at a site is not readily displaced by another ColE1 plasmid in the cytoplasm. Before cell division, the replication site or sites are duplicated and occupied by plasmid molecules that are the direct descendents of the plasmids occupying the parent sites. Any two ColE1 derivatives are incompatible because only a single plasmid molecule has access to each replication site.

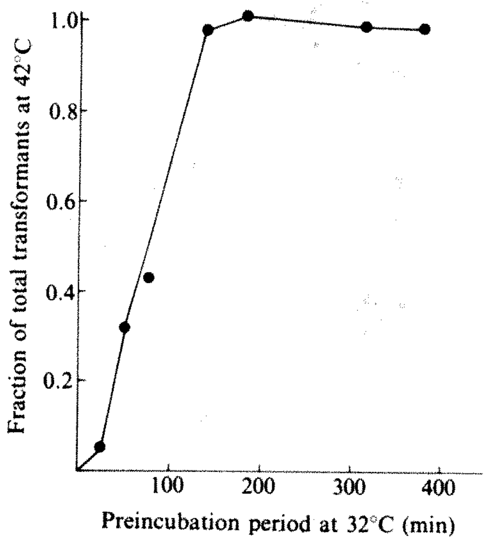

Fig. 3 Growth of pJB28 transformants at 32 °C and 42 °C. pJB28 was constructed by ligation, *in vitro*, of *Eco*RI linearised pJC307 with the *Eco*RI fragment of pML21 containing the genes for Km^r. Purified pJB28 plasmid DNA at a concentration of 5 μg ml^{-1} was used to transform HB101 as described in Fig. 1 legend except that the heat pulse was at 32 °C and the cells were not grown in broth before plating. Aliquots of the transformation mixture were grown on LB agar plates containing 10 μg ml^{-1} Km at 32 °C and, at the indicated times, transferred to 42 °C. Colonies were counted after 24 h of growth at 42 °C. As a control, several plates were incubated at 32 °C only.

In its simplest form, this site model predicts that *E. coli* contains a single site for ColE1 plasmid propagation. The results presented above indicate that both pMB9 and pML21 replicate in primary transformants. To reconcile this result with a single site replication model, it is necessary to propose that some fraction (about 1–5% based on Fig. 1) of a logarithmically growing population of *E. coli* cells transitorily contain two sites for ColE1 propagation. Such cells may have duplicated the single site but not undergone cell division at the time of transformation by plasmid DNA. Double transformants could arise if pMB9 occupies one and pML21 occupies the other site of such cells. Both plasmids could then replicate within this single cell until the point at which cell division occurs and the two sites are segregated. After cell division pMB9 continues to be replicated and propagated in one of the daughter cells and its progeny, and pML21 in the other daughter cell and its progeny. The random segregation of the non-site bound copies of both plasmids and their protein products enables the transitory maintenance of the double transformant phenotype.

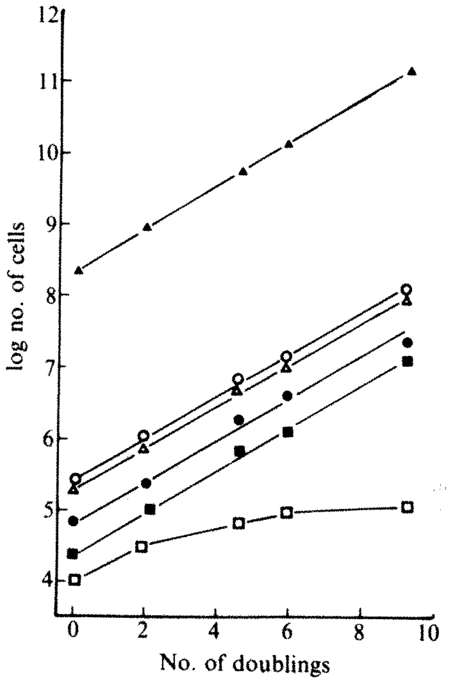

Fig. 4 Kinetics of segregation of pMB9 and pML21 from pMB9–pML21 double transformants. pJB56 was constructed by joining a derivative of RSF1030 containing an *Eco*RI site with the *Eco*RI fragment of pML21 containing the gene for Km^r. Mixtures of pMB9 and pJB56 DNAs or mixtures of pMB9 and pML21 DNAs (all at 20 μg ml^{-1}) were incubated with 10^8 CaCl$_2$-treated HB101 cells. After incubation on ice for 60 min, the cells were heated to 42 °C for 2 min, diluted 10-fold in LB, and incubated at 37 °C for 20 min. Aliquots of transformed cells were plated on broth plates and on plates containing 10 μg ml^{-1} Tc, 20 μg ml^{-1} Km, and 10 μg ml^{-1} Tc + 20 μg ml^{-1} Km. The remaining cells were diluted fivefold in LB and grown for two doublings at which time the plating was repeated. This last procedure was repeated several times to give about 10 generations of growth. Plates were incubated at 37 °C for 30 h and all visible colonies were counted. Total cells (▲), pMB9 (○), pML21 (△), pJB56 (●), pMB9 + pJB56 (■), pMB9 + pML21 (□).

Alternatively, *E. coli* may contain more than one replication site for ColE1 plasmids and double transformants could arise simply by two different plasmids acquiring one of the multiple sites. For a multiple site model to be in keeping with our data it is necessary to assume that the sites are segregated in a linear directive fashion. On cell division each site is duplicated, maintaining the linear arrangement with respect to one another. Half the linear array of sites are segregated to one daughter cell and half are segregated to the other daughter cell.

We present additional results below which support a replication site model as the basis of ColE1 incompatibility.

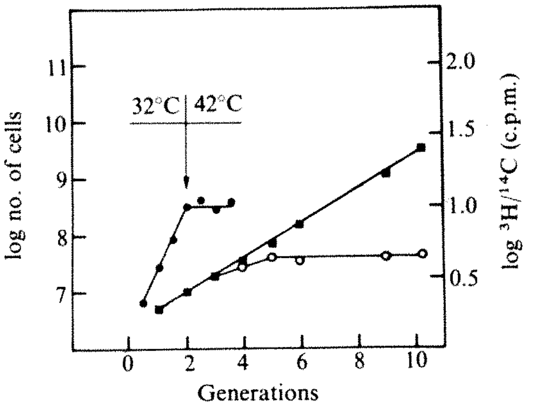

Fig. 5 Kinetics of segregation of pJB28 plasmid. *E. coli* strain FMA119 ($F^- su^- gal^- Sm^R recA^- thyA^- deo^-$) containing the temperature-sensitive replication plasmid pJB28 was grown in LB at 32 °C or 43 °C through ~10 doublings of the total population. Logarithmic growth was maintained throughout by successive dilution of the culture. At times indicated, aliquots of the culture were removed and plated on LB agar containing Km (20 μg ml^{-1}). In a separate experiment, FMA119 cells containing pJB28 were grown in 50 ml of minimal media supplemented with 2 μg ml^{-1} thymine and 20 μg ml^{-1} ^{3}H-thymine. Cells were grown for 2 doublings at 32 °C in the presence of the label then diluted by a factor of 5 in the same media and grown at 43 °C for 2 doublings. At the times indicated 10-ml samples were removed and mixed with equal aliquots of FMA119 cells grown at 32 °C containing pJB28 which had been labelled with ^{14}C-thymine. The cells were pelleted, washed in 10 ml cold 10 mM Tris-HCl, *p*H 8.0, 1 mM EDTA and resuspended in 0.2 ml 20% sucrose containing 10 mM Tris-Hcl *p*H 8.0. the cells were incubated with lysozyme (2 mg ml^{-1}) for 5 min at 4 °C, 0.25 ml 0.25 M EDTA was added, the mixture was incubated at 4 °C with occasional gentle mixing, 0.25 ml of lysis buffer containing 1% Brij 58, 0.4% sodium deoxycholate, 6.25 mM EDTA and 10 mM Tris-HCl *p*H 8.0 was added, and the mixture was incubated at 45 °C for 2.5 min. The lysed cells were centrifuged at 17,500 r.p.m. for 60 min at 4 °C in a Sorvall SS34 rotor in an RC2B centrifuge. The supernatant solution was extracted with equal volumes of phenol and chloroform. After an additional chloroform extraction, the aqueous phase was precipitated with ethanol. The precipitate was washed with 65% ethanol, dried, resuspended in 0.2 ml H_2O and mixed with 1 μg purified pJB28 DNA. The samples were applied to 1% agarose tube gels (1 cm × 12 cm) and electrophoresed at 3 mA per gel for 14 h. Electrophoresis conditions, gel staining and photography were as described by Bedbrook and Bogorad[17]. the fluoroescence corresponding to supercoiled pJB28 DNA was sliced from the gel, soaked in aquafluor for 16 h, and assayed in a liquid scintillation counter. The radioactivity incorporated into pJB28 DNA at 32 °C and 43 °C are superimposed with the results obtained for pJB28 segregation at 32 °C and 43 °C. ●, Ratio of ^{3}H c.p.m. in pJB28 DNA to the standard amount of ^{14}C c.p.m. in pJB28 DNA. ■, Number of cells plating on Km plates after growth at 32 °C. ○, Number of cells plating on Km plates after growth at 42 °C.

The kinetics of segregation of double transformants

Figure 4 illustrates experiments in which the kinetics of the segregation of pMB9-pML21 double transformants were compared with those of pMB9-pJB56 (a Km^r-conferring derivative of RSF1030) double transformants. Double transformants were constructed as described in Fig. 1 legend. The total cell population used in the transformation was then grown logarithmically without selection for about 10 doublings. At various times, samples were plated to assay total cell number and the presence of the two plasmids. The fraction of the total population transformed by pMB9 or pML21 was about 10^{-3} and the fraction transformed by pJB56 was about 2×10^{-4}. The number of pMB9-pML21 double transformants was about 10% that of the single transformants. This number is likely to be an underestimate of the absolute number of primary double transformants as some segregation must occur during the unselective growth period before the first plating. The total cell population grew logarithmically throughout the experiment and no loss by segregation of single pMB9 or pJB56 transformants or double pMB9 + pJB56 transformants was observed after 10 generations. pMB9–pML21 double transformants increased initially, in a non-log-linear fashion, to give ~10 times the number of "doubly transformed" cells. After six doublings of the total cell population, the number of pMB9–pML21 double transformants had plateaued. These data corroborate the experiments described above which showed that pMB9–pML21 double transformants are unstable and can only exist for short periods of time.

Figure 5 shows that the kinetics of segregation of pMB9–pML21 double transformants are similar to the kinetics of distribution of pJB28 to daughter cells following a shift to the nonpermissive temperature for pJB28 replication. At 32 °C, both the growth of Km^r cells and the replication of pJB28 (as shown by ^{3}H-thymidine incorporation in plasmid DNA) are log-linear. After transfer to 42 °C, plasmid DNA synthesis stopped within the temperature shift and the next measurement of plasmid synthesis (20 min). In contrast, the number of Km^r-resistant cells increased in a non-linear fashion during the first four doublings of the total cell population at 42° C, at which point the number of Km^r cells plateaued.

Comparison of Figs 4 and 5 shows that the extent of growth of pJB28-containing cells following a shift to 42 °C is similar to the increase in pMB9–pML21 double transformants immediately following transformation. This relationship suggests that the 10-fold increase in pMB9–pML21 double transformants was due to the distribution to daughter cells of pML21 and pMB9 plasmids which were produced in the primary transformed cell. These kinetic observations are consistent with a site model of plasmid replication because they indicate extremely rapid cessation of replication of one of the two plasmids in the daughter cells of the primary transformant. This leads rapidly to a steady state condition in which there is no net increase in double transformants. Moreover, the 'double transformants' obtained are genetically unstable and cannot be maintained even by selection. These observations are not consistent with a

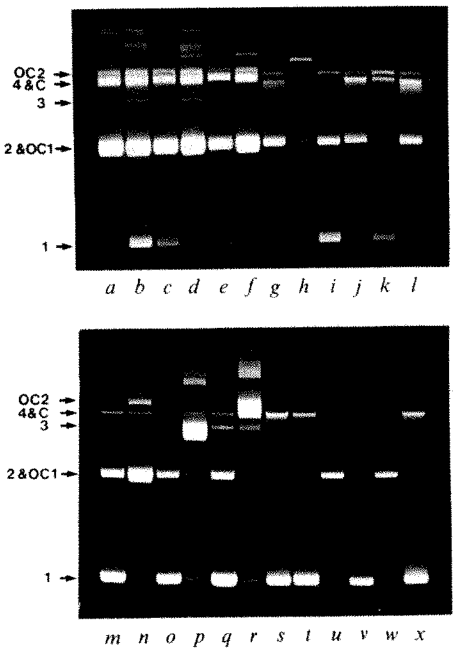

Fig. 6 *E. coli* propagates only one multimer form of pMB9 per cell. *E. coli* strains JC1157 and JC1157 *rec*A ts (ref. 16) were transformed with pMB9 monomer DNA as described in Fig. 4 legend. Transformed cells were plated at 32 °C on LB agar containing 10 μg ml^{-1} Tc. Cells from colonies of JC1157 (pMB9) and JC1157 *rec*A ts(pMB9) grown at 32 °C were plated again at 32 °C and 42 °C. Six colonies from each of the four platings were used to prepare plasmid DNA as described in Fig. 5 legend. Plasmid DNA samples were electrophoresed in 1% agarose gels. Electrophoresis is from top to bottom. pMB9 DNA from various colonies of JC1157 grown at 32 °C (lanes *a–f*), from JC1157 grown at 42 °C (lanes *g–l*), from JC1157 *rec*A ts grown at 32 °C (lanes *m–r*), and JC1157 *rec*A ts grown at 42 °C (lanes *s–x*). pMB9 supercoiled monomers (1), pMB9 supercoiled dimers (2), pMB9 supercoiled trimers (3), pMB9 supercoiled tetramers (4), pMB9 open circular monomers (OC1), pMB9 open circular dimers (OC2), and chromosomal DNA (C).

model which invokes copy number repressors as the basis for plasmid incompatibility because repressor models predict an indefinite log-linear increase in the number of double transformants. With a positive selection for both plasmids one expects to maintain an increasing lineage of cells containing both plasmids[6], even in an extreme case in which two incompatible plasmids have very different affinities for the replication repressor.

Transformation of cells containing resident plasmids

Table 2 lists the transformation frequencies obtained when pMB9 was used to transform HB101 containing various resident plasmids. In this experiment the transformation frequency of HB101 by pMB9 was 10^{-5} per cell. This frequency was reduced by more than three orders of magnitude when HB101 contained pML21 but was unaffected by the residency of pJB56, a compatible plasmid. Residency by pJB55, a hybrid replicon between RSF1030 and pMB9, reduced the transformation frequency by pMB9 to a similar extent as that caused by pML21, but residency by pMB9 did not affect the transformation frequency of pJB55.

These data can be explained by a site model for incompatibility. As discussed above, the exchange of plasmids at the replication site is postulated to be an infrequent event. Thus, the accessibility of the site to an incoming plasmid would be greatly reduced if the site were already occupied by an incompatible resident plasmid. The hybrid replicon pJB55 could reside at both the sites for ColE1 and RSF1030 replication. When in residence, pJB55 would be expected to prevent pMB9 from acquiring the site. On the other hand, the hybrid plasmid should be able to acquire the RSF1030 site when pMB9 is the resident plasmid.

We have found that pMB9–pJB55 double transformants are propagated stably and indefinitely. This result is not consistent with a replication repressor model for plasmid incompatibility as repressor synthesised by the ColE1 portion of the RSF1030–ColE1 hybrid replicon would be expected to induce the segregation of pMB9.

Physical evidence for a directive segregation model

Experiments shown in Fig. 6 provide physical evidence that *rec*A$^-$ *E. coli* is capable of replicating only a single ColE1 plasmid per cell. for this experiment we took advantage of the following: (1) pMB9 plasmid molecules undergo reciprocal recombination in *rec*$^+$ cells to form plasmid multimers at high frequency[14]. In contrast, multimer forms are stably propagated in *rec*A$^-$ *E. coli.* (2) A temperature sensitive (ts) *rec*A$^-$ mutant of *E. coli* forms plasmid multimers when the plasmid-containing strain is grown at 32 °C but not at 42 °C.

In the experiment shown in Fig. 6, the fate of plasmid multimers formed and present in the cells of the ts *rec*A$^-$ strain grown at 32 °C, was followed when the temperature was shifted to 42 °C. As a control, *rec*$^+$ strain, otherwise isogenic with the ts *rec*A$^-$ strain was used. Cultures growing at 32 °C were plated for single colonies at 32 °C and 42 °C and plasmid DNAs derived from single colonies were examined by gel electrophoresis. For the *rec*$^+$ strain (Fig. 6*a–l*), six colonies analysed at 32 °C and 42 °C all contained different multimer forms. Various multimers were also seen in DNA preparations from the ts *rec*A$^-$ colonies plated at 32 °C (Fig. 6*m–r*). When the ts *rec*A$^-$ strain was plated at 42 °C, however, individual colonies contained only one plasmid size class (Fig. 6*s–x*): four contained monomers and two contained dimers. That is, the ts *rec*A$^-$ strain propagated only one of the possible size classes of pMB9 plasmid molecules at 42 °C despite the fact that the cells growing at 32 °C contained various multimer forms.

Table 2 Effect of resident plasmids on transformation frequency

Transforming plasmid	Resident plasmid	Transformation frequency per cell
pMB9	None	10^{-5}
pMB9	pML21	$<10^{-8}$
pMB9	pJB56	10^{-5}
pMB9	pJB55	$<10^{-8}$
pML21–RSF1030 hybrid (pJB55)	pMB9	10^{-6}

Strain HB101 containing the indicated plasmids was grown in LB media containing 20 μg ml^{-1} Km or 10 μg ml^{-1} Tc as appropriate. Approximately 10^9 cells were transformed with 1 μg of the indicated plasmid DNAs as described in Fig. 1 legend. Transformants were grown in LB media containing 10 μg ml^{-1} Tc or 10 μg ml^{-1} Km to select the resident plasmid and then plated on LB solid medium containing 10 μg ml^{-1} Tc and/or 20 μg ml^{-1} Km to select both the resident and the incoming plasmid. The transformation frequency indicated is the number of cells containing both resident and incoming plasmids divided by the total number of cells used in the transformation.

Discussion

We have presented evidence that strongly supports the view that incompatibility between ColE1 plasmids is a direct consequence of competition between plasmids for a single or a limited number of plasmid replication site(s). Our results contrast other studies[6,8] which show that *E. coli* strains are capable of maintaining two ColE1 plasmid replicons over many generations. The results of these studies are best explained if plasmid incompatibility is envisaged to be the consequence of transacting repressors which normally regulate plasmid copy number. A significant difference in the experimental design of these previous studies and the experiments described here is that we have used *rec*A$^-$ strains. We have shown previously that reciprocal recombination occurs between plasmid molecules in *rec*$^+$ (but not in *rec*A$^-$) *E. coli* leading to the formation of plasmid multimers[14]. Recombination occurs only at regions of homology and is reversible. We showed that stable double transformants can be obtained when pMB9 and pML21 are used to simultaneously transform a *rec*$^+$ strain. These doubly transformed strains contain hybrid pMB9–pML21 plasmids[14].

The phenomenon of plasmid–plasmid recombination can be invoked to reconcile our observations with previous studies. Assume that a *rec*$^+$ strain is replicating a pMB9–pML21 hybrid plasmid (formed *in vivo* by recombination) at a ColE1 replication site. Occasionally, intramolecular recombination of the site bound hybrid plasmid will occur and will result in occupation of the site by pMB9 or pML21 instead of the pML21–pMB9 hybrid. From this point on, the site will only replicate pMB9 or pML21 and one of the two plasmids may be segregated away unless it regains access to the replication site by recombination.

Warren and Sherratt[6] found that smaller ColE1 plasmids with high copy numbers usually displaced larger, low copy number plasmids. This observation can also be explained by plasmid–plasmid recombination. Assume that a hybrid ColE1 replicon is comprised of two plasmids, which, as monomers, are present in different copy numbers. Following intramolecular recombination and cell division, daughter cells carrying the 'high copy' replicon at the replication site will contain a high ratio of 'high copy' monomers to hybrids. In comparison, daughter cells which contain the 'low copy' replicon at the replication site, will contain a lower ratio of monomers to hybrids. The higher the ratio of monomers to hybrids, the lower the probability that the replicating monomer at the site will recombine with a hybrid plasmid to restore the double transformant genotype. A similar argument can be made for large versus small plasmids. A small site bound plasmid would have less extensive homology with the hybrid plasmids in the cytoplasm than would a large site-bound plasmid. This decreases the probability that a small site-bound plasmid would recombine with a cytoplasmic hybrid plasmid, increasing the probability that the small plasmid would displace the larger one. That is, recombination may be the basis of incompatibility hierarchies among ColE1 replicons[6]; if so such hierarchies would not be expected to be observed in *rec*A$^-$ strains.

We conclude that plasmid incompatibility is the consequence of ordered, directive segregation of replication sites. For pMB9 and pML21 plasmids attachment to the replication site seems to be irreversible. Certain ColE1 plasmid derivatives appear to segregate from *E. coli* strains spontaneously (Ausubel and Ow, unpublished). Presumably such plasmids have an altered affinity for the replication site, compared with say pMB9, such that the binding with the site is unstable. For these plasmids the affinity for the replication site and copy number control would be important in controlling incompatibility. Take for example a primary double transformant *rec*A$^-$ cell containing a 'stable' attachment plasmid at one site and an 'unstable' attachment plasmid at another site; daughter cells sufficiently distant in the cell lineage from the primary cell to have completely segregated the replication sites may still contain non-site-bound plasmids of both types. If at this point the 'unstable' attachment plasmid was uncoupled from a replication site exchange with the stable attachment plasmid may occur thus switching the plasmid 'genotype' of the cell. Hierarchies of incompatibility could be generated by variable affinities of various plasmids for the replication sites.

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Three new types of viral oncogene of cellular origin specific for haematopoietic cell transformation

M. Roussel, S. Saule, C. Lagrou, C. Rommens, H. Beug*, T. Graf* & D. Stehelin†
INSERM, Oncologie Moléculaire, Institut Pasteur de Lille, 15 rue C. Guérin F 59000 Lille, France

*Institut für Virusforschung, Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 280, D6900 Heidelberg, FRG

The RNAs of seven replication-defective leukaemia virus (DLV) strains contain three types of unique sequences, which correlate with the capacity of a given virus strain to transform erythroblasts, macrophage-like cells and myeloblasts, respectively. These sequences, termed erb, mac *and* myb, *have their counterparts in the normal DNA of avian and mammalian species. Our results indicate that DLVs represent recombinants between a common 'vector' related to a chicken endogenous virus and one of three types of cellular gene possibly involved in haematopoietic differentiation.*

ON the basis of their oncogenic properties, avian retroviruses are assigned to either of two major classes. Of many characterised natural field isolates, most (non-defective avian leukaemia viruses, or ALVs) are 'weakly oncogenic' and cause predominantly lymphatic leukaemia after a latency period of months to years following inoculation into susceptible strains of chickens. The ALVs most frequently occur as independent agents containing all the genetic information required for viral replication. In contrast, several avian retroviruses are highly oncogenic, causing disease after a latency period of days to weeks. These include the replication-competent avian sarcoma viruses (ASVs, or Rous sarcoma viruses) which readily induce sarcomas in susceptible birds and transform fibroblasts *in vitro*, and also the replication-defective leukaemia viruses (DLVs) which require helper ALVs for propagation in cultured cells (for review, see ref. 1).

The seven available independent isolates of DLVs have been assigned to three subgroups based on the types of neoplasm they induce and on the differentiation phenotypes of haematopoietic cells they transform *in vitro*[1,2]. (1) The avian erythroblastosis-type viruses (AEV strains R and ES4 are probably identical[3]) induce erythroblastosis *in vivo* and transform erythroblasts *in vitro*. (2) The avian myelocytomatosis-type viruses (MC29, CMII, OK10 and MH2) transform macrophage-like cells *in vitro* and some strains induce myelocytomatosis *in vivo*. (3) The avian myeloblastosis-type viruses (AMV and E26) induce myeloblastosis *in vivo* and transform myeloblasts *in vitro*. AEV can also induce sarcomas and transforms fibroblasts *in vitro*, and MC29-type viruses can induce carcinomas and occasional sarcomas and transform epithelial cells and fibroblasts *in vitro*[1,2]. In this report we have investigated whether the oncogenicity of these viruses could be related to the presence of specific nucleotide sequences in their genome. Such a relationship would imply that part or all of these specific sequences represent new viral oncogenes.

DLVs are related to non-defective avian leukaemia viruses

DLVs were originally isolated from chickens in association with their natural replication-competent helper viruses belonging to the ALV group. Attempts to complement DLVs with helpers unrelated to ALVs have failed[4]. A possible homology between certain genomic sequences of the DLVs and ALV genes[5–7] was

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† To whom correspondence should be addressed.

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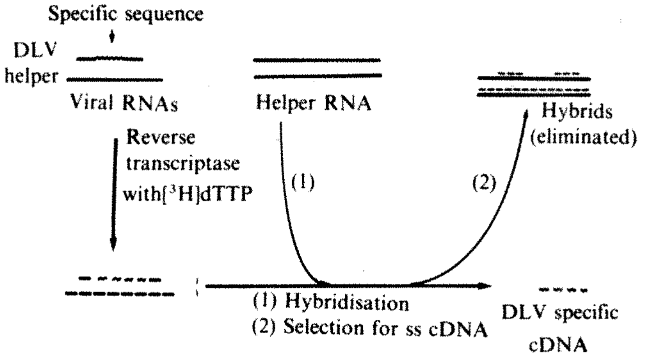

Fig. 1 Scheme for the preparation of DLV-specific cDNAs. Cloned AEV (RAV-2) and MC29 (RAV-2) pseudotypes, obtained by rescuing these viruses with RAV-2 from cloned nonproducer fibroblasts, were propagated in chicken fibroblasts. Viral RNAs were extracted from the pelleted viruses, then transcribed into labelled cDNA using *in vitro* reverse transcription with purified AMV reverse transcriptase, ^{3}H-dTTP and unlabelled dCTP, dGTP, dATP and calf thymus priming. In a negative selection step each cDNA mixture was hybridised to an excess of RNA extracted from RAV-2 grown separately. Single-stranded DLV-specific cDNAs were then isolated after fractionation on hydroxyapatite. AMV-specific cDNA was prepared in a similar way, but using RNA of AMV (helper) pseudotype obtained from the plasma of infected chickens, and a mixture of ALV helper RNAs extracted from RAV-1, RAV-2 and MAV-2 (0) viruses for the selection. Non-hybridisable radioactivity was eliminated from the cDNAs by a positive selection with homologous RNAs[8] (not shown). Specific activities of the ^{3}H-cDNAs were $\sim 50 \times 10^6$ c.p.m. per μg. Details of these experiments will be described elsewhere.

investigated by testing clones of avian cells containing the DLV provirus without its helper. Such cells, usually unable to produce virus[6], still contain several RNA transcripts of the DLV provirus. We tested whole RNA of such nonproducer cells, using molecular hybridisation with labelled probes representing a homogeneous transcript of an ALV, td Pr-B (cDNArep), or of the *src* gene of ASVs (cDNAsarc)[8]. Table 1 indicates that all the DLV genomes tested contain ALV-related nucleotide sequences in different amounts (28–77%), but lack the *src* gene of ASV[9,10]. However, the detected homology to ALV cannot account for the entire genetic content of the DLVs, which all contain 6,000–8,000 nucleotides in their 28–34S genome[11,12]. Thus, these viruses may contain sets of nucleotide sequences unrelated to ALVs.

DLV-specific sequences

We looked for specific sequences by analysing the prototype strain of each of the three DLV subgroups, namely AEV, MC29 and AMV. Clone viral strains were obtained for AEV and MC29 by superinfection of nonproducer cells with the plaque-purified helper virus RAV-2. The rescued viruses were then tested for their oncogenic potential *in vitro* (using the bone marrow transformation assay[2]) and propagated in chicken erythroblasts (for AEV) or chicken fibroblasts (for MC29). AMV was obtained in high yields from the plasma of infected chickens.

The preparation of specific cDNAs of these viruses (Fig. 1) was similar to the selection of cDNA specific for the *src* gene of ASVs[8]. cDNAs of AEV, MC29 and AMV were selected so that they could still hybridise to their RNAs of origin, but not to the RNA of the helper viruses used to grow the viral stocks or any of the other ALVs tested. These specific cDNAs were denoted cDNAaev for AEV, cDNAmc29 for MC29 (ref. 13) and cDNAamv for AMV.

The distribution of viral nucleotide sequences related to these specific cDNAs was explored by hybridising them at plateau C_rt values to several viral RNAs. The results (Table 2) allow several conclusions to be drawn. First, the cDNAs tested are specific for the DLVs used for their preparation. Second, they represent distinct sets of sequences, unrelated to each other by cross-hybridisation experiments, and third, cDNAmc29 shows extensive homology to RNA of CMII, OK10 and MH2, as does cDNAamv to E26 RNA. As will be described elsewhere, the three cDNAs exhibited exactly the same specificity when tested with cellular RNAs extracted from nonproducer cells, further demonstrating that the probes indeed detect sequences specific for the three types of DLV.

Table 1 DLV genomes in nonproducer cells are related to ALVs

DLV strain	Type of nonproducer cell	Annealing (% S_1 resistance) with cDNArep	cDNAsarc
AEV	chicken fibroblast	28	<3
MC29	quail fibroblast	45	<3
CMII	quail fibroblast	41	<3
OK10	quail fibroblast	77	<3
MH2	quail fibroblast	32	<3
AMV	chicken myeloblast	50	<3
E26	chicken myeloblast	36	<3

Total RNA was extracted from the different avian nonproducer cells and hybridised in stringent conditions (25-μl aliquot containing 1–10 mg ml^{-1} RNA, 0.6 M NaCl, 68 °C) with cDNAs (1,000 c.p.m. per point = 0.02 ng) to plateau C_rt values (defined as 10× the $C_rt_{1/2}$). cDNArep and cDNAsarc were prepared as described earlier[3], but using calf thymus-primed polymerase reactions[14]. Hybrids were assayed by S_1 nuclease. No correction for the expression of endogenous RAV-O or cellular sarc was made as they gave values below 3% in each case.

To investigate the origin of the DLV-specific sequences, DNA extracted from normal avian cells was tested for its ability

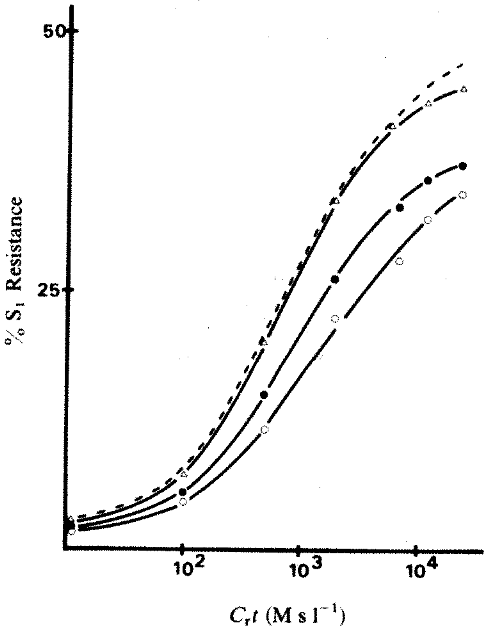

Fig. 2 DLV-specific sequences have their couterparts in normal chicken DNA. Aliquots of 11-day chicken embryo DNA (200 μg per point, sheared to 4–6S) were hybridised with the different specific cDNAs (1,000 c.p.m. per point = 0.02 ng) in stringent conditions (0.6 M NaCl, 68 °C) at increasing C_0t values. Hybrids formed were tested for their resistance to S_1 endonuclease. ●—●, cDNAaev; △—△, cDNAmc29, ○—○, cDNAamv; ----cDNAsarc. Similar results were obtained with normal quail embryo DNA (not shown).

Table 2 DLVs contain unique nucleotide sequences

RNA derived from	Annealing (% S_1 resistance) with cDNAaev	cDNAmc29	cDNAamv
AEV	100	<3	<3
MC29	<3	100	<3
CMII	<3	96	<3
OK10	<3	91	<3
MH2	<3	66	<3
AMV	<3	<3	100
E26	<3	<3	68
ALV helpers (control)	<3	<3	<3

Viral 50–70S RNAs were extracted and hybridised to plateau C_rt values ($>10\ \mathrm{M\,s\,l^{-1}}$) in stringent conditions (0.6 M NaCl, 68 °C) with the specific cDNAs (1,000 c.p.m. per point = 0.02 ng). The hybrids formed were tested for S_1 nuclease resistance. Results obtained were standardised to the values of the homologous reaction. Maximum hybridisation of cDNAaev with AEV RNA was 95%; that of cDNAmc29 with MC29 RNA 90% and that of cDNAamv with AMV RNA 95%. The DLVs tested were cloned pseudotypes with RAV-1 or RAV-2 helper ALV, with the exception of AMV and E26, which were not cloned. The ALV helper RNAs tested as controls were obtained from RAV-1, RAV-2, RAV-49, RAV-50, MAV-2 (0). The numbers boxed indicate the important homologies.

to hybridise with cDNAaev, cDNAmc29 and cDNAamv. Figure 2 shows that all three types of sequences have their counterparts in normal cellular chicken DNA and, as judged from the $C_0t_{1/2}$ values, that they are present in the non-repetitive DNA at one or two copies each per haploid genome. The low plateau values observed in Fig. 2 are due to the competition of DNA reassociation with the DNA–cDNA hybridisation reaction; transcription analyses (see below) show that most or all of the specific sequences are present in normal chicken DNA. Results obtained with molecular hybridisation in liquid phase in stringent conditions show that the bulk of the non-repetitive chicken DNA sequences have been lost relatively rapidly during evolution: labelled chicken unique sequence DNA barely anneals (Fig. 3) to the DNA of species from another genus. For example, quail and chicken, which probably evolved from a common ancestor some 20–40 Myr ago, have DNAs that share less than 25% homology when cross-hybridised (Fig. 3). Similarly, ALV-related 'virogene' sequences have recently been shown to be absent in some species of the Galliformes[14]. In contrast, cellular sequences related to cDNAaev, cDNAmc29 and cDNAamv have been highly conserved throughout the phylogeny of the higher vertebrates. As also shown in Fig. 3, their 'evolutionary half lives' are comparable to those of known stable genes such as globin or ovalbumin. The latter values resemble thoses obtained earlier for the transforming gene *src* of ASVs[15–17] and agree with the results recently described for MC29 by Sheiness *et al.*[18].

As normal cells contained nucleotide sequences related to highly transforming viruses, we studied the rate of transcription of such sequences. Total RNA extracted from normal chicken (or quail) fibroblasts was hybridised to the different specific cDNAs. Figure 4 shows that these sequences are all transcribed at a low level (a few copies per cell), as previously found for the expression of "cellular sarc" (refs 16, 19). Cellular sequences related to cDNAaev are transcribed at two or three copies per cell; cellular sequences related to cDNAmc29 are reproducibly expressed at a higher level (5–10 copies per cell), as Sheiness *et al.*[18] found. In contrast, cellular sequences related to cDNAamv are transcribed at lower levels in fibroblasts (one copy per cell). As can be seen in Fig. 4, all these cellular sequences reach plateau hybridisation values close to 100%, and must therefore be essentially fully represented in normal cellular nucleic acids.

Three new oncogenes

Our results show that all seven defective leukaemia viruses studied contain part or all of one out of three different sets of specific nucleotide sequences complementary to cDNAaev, cDNAmc29 or cDNAamv, which have their counterpart in normal cells of higher vertebrates. None of these sequences is related to the transforming gene *src* of ASVs. Although it is not understood how recombinants between ALV sequences and cellularly derived sequences can acquire oncogenic capacity, several indications favour the hypothesis that three specific transforming genes, termed *erb*, *mac* and *myb*, are detected by cDNAaev, cDNAmc29 and cDNAamv, respectively. (1) There is a strict correlation between the presence of *erb*, *mac* or *myb* sequences in a given virus and its selective capacity to generate transformed erythroblasts, macrophage-like cells or myeloblasts[1,2,20]. (2) The properties of these sequences and their origin are reminiscent of results obtained earlier for the transforming gene *src* of ASVs[15]: size, cellular origin and phylogenetic stability[16,17]. (3) As indicated by heteroduplex mapping studies with MC29 (ref. 21), these sequences do not appear as bits of sequences randomly distributed throughout the viral RNA, but rather as continuous stretches located inside the viral genome. (4) Temperature-sensitive mutants have recently been isolated from AEV, indicating that a virus-coded protein must be synthesised to maintain the undifferentiated state of transformed erythroblasts[22]. (5) All DLVs tested contain the 5′ region of the ALV genome (partial *gag* gene[12]). In the strains studied so far (MC29 and MH2) this region was located adjacent to the DLV-specific sequence[21,23]. In addition, cells nonproductively transformed by AEV, MC29, CMII and MH2 express novel polyproteins of molecular weight 75,000 (ref. 3), 110,000 (refs 6, 7), 90,000 (ref. 7) and 100,000 (ref. 4), respec-

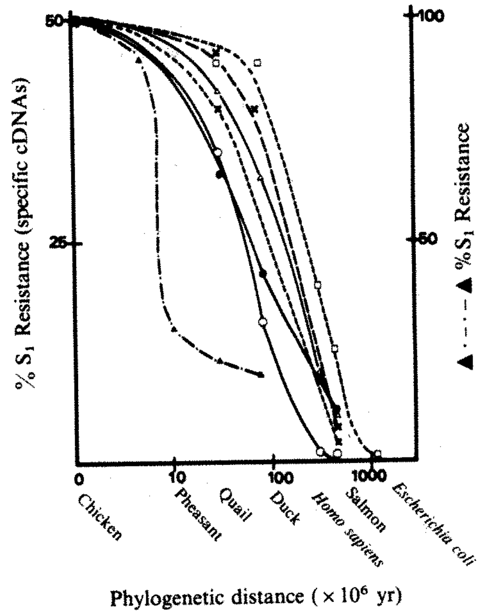

Fig. 3 Stability of cellular DLV-related specific sequences during evolution. Aliquots of DNA extracted from different species having diverged increasingly long ago with speciation were hybridised in the specific conditions described in Fig. 2. The values obtained were standardised arbitrarily, for comparison purposes, to 50% for the chicken, which was also taken as the origin of the scale indicating phylogenetic distance. ●—●, cDNAaev; △—△, cDNAmc29; ○—○, cDNAamv; □- - -□, cDNAsarc. Also shown (▲ · — · —▲) are results obtained with ^{3}H-labelled chicken unique sequence DNA[14](1,000 c.p.m. per point = 20 ng) in similar conditions (standardised to 100% for homologous reassociation) and with cDNAs representing genes known to be conserved throughout the evolution of vertebrates; ×— —×, cDNAlgo (ref. 16) for globin; ×- - - - -×, cDNAova (ref. 16) for ovalbumin.

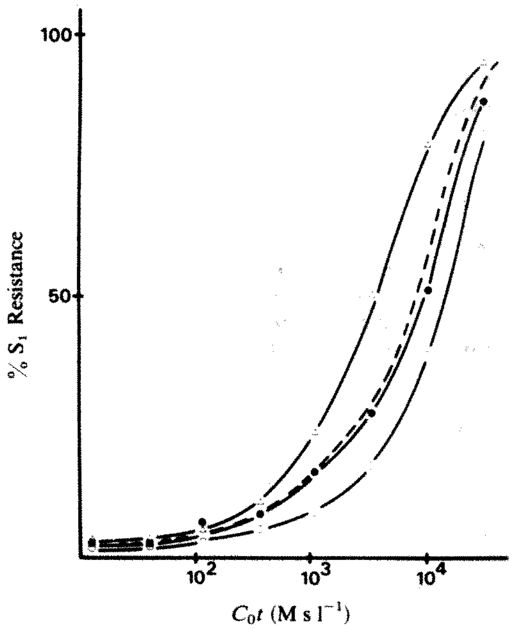

Fig. 4 Transcription of the cellular DLV-related specific sequences in normal fibroblasts. Total RNA was extracted from normal chicken embryo fibroblasts and hybridised at increasing C_rt values (varying RNA concentration up to 10 mg ml^{-1}) in stringent conditions (0.6 M NaCl, 68 °C), to the specific cDNAs (1,000 c.p.m. per point = 0.02 ng). Hybrids were scored for their S_1 endonuclease resistance. Symbols are as in Fig. 2.

tively, with the structure: partial *gag-x* or *y*, where *x* or *y* represent polypeptide moieties which are antigenically unrelated to known ALV proteins, and presumably correspond to part or all of the specific *erb* or *mac* nucleotide sequences. That these polyproteins could be involved in the transformation process is indicated by the finding that the type of tryptic peptide pattern exhibited by the non-*gag* portion of the polyprotein of a given DLV closely correlates with the transformation specificity of the virus (M. J. Hayman, personal communication, and ref. 24). In their general structure and ability to code for *gag*-related polyproteins, avian DLVs resemble replication-defective mammalian retroviruses capable of inducing leukaemias or sarcomas with a short period of latency, such as the Abelson[25] murine virus and feline sarcoma virus[26,27].

Avian DLVs behave like recombinants between an ALV-related vector virus of low oncogenicity and unknown origin[14] and an evolutionarily stable set of nucleotide sequences of cellular origin. In this regard they also closely resemble ASVs (for review, see ref. 11). The mechanism of such a recombination remains to be elucidated, but could be related to the jumping polymerase hypothesis proposed recently by Coffin[28]. How frequently such a recombination occurs is not known, although about 30 DLV-like and 1–3 ASV-like independent isolates have been reported[2]. However, a transduction of cellular sequences would not necessarily lead to such severe effects as the generation of a highly oncogenic virus and may therefore be much more frequent than anticipated. Indeed, we have recently found in an ASV stock a new virus of unknown oncogenicity containing apparently unknown cellular genetic material (N. Pluquet and D.S., unpublished).

That only a partial homology was observed between the specific sequences of MC29 and MH2 as well as between the specific sequences of AMV and E26 (Table 2) can be explained in three ways—these viruses acquired cellular genes which already differed in their sequences; they acquired the same gene and the observed differences reflect a divergence introduced by passage of the virus; a second recombination event occurred in MH2 and E26. The finding that there are only one or two copies per haploid genome of these genes present in cellular DNA is consistent with the second possibility. The observation that there is a somewhat lower degree of homology of cDNAamv to cellular DNA than that of the other types of specific cDNA tested (Fig. 2) might reflect the long passage history of AMV and would also agree with the second of the above interpretations. But hybridisation experiments between cDNAmc29 and MH2 RNA carried out in non-stringent conditions do not show the significant increase of annealing which the second interpretation would predict (M.R., S.S. and D.S., unpublished) and thus leave that question unanswered.

The role of DLVs in leukaemic transformation could be explained by a generalisation of the 'differentiation block' hypothesis[22,24]: the oncogenes of DLVs are homologous to normal cellular genes ('cellular *erb*, *mac* or *myb*') coding for lineage-specific haematopoietic differentiation proteins; the transforming proteins act by competitively inhibiting the corresponding cellular proteins[2,17], thus leading to a block of differentiation and subsequent leukaemogenesis. This hypothesis would predict that RNA sequences corresponding to the cellular *erb*, *mac* and *myb* genes are preferentially expressed in normal erythroblasts, macrophages and myeloblasts, respectively. Spontaneous leukaemogenesis may then simply result from a malfunction of such genes. Indeed, several different genes may be able to induce the same type of leukaemia. In this respect, it would be interesting to know whether there are viruses causing the same type of leukaemia but containing different cell-related oncogenes. Such viruses would also be useful for elucidating why the DLVs also induce sarcomas or carcinomas, an additional specificity for non-haematopoietic tissues difficult to explain by the differentiation block theory.

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T4 DNA topoisomerase: a new ATP-dependent enzyme essential for initiation of T4 bacteriophage DNA replication

Leroy F. Liu, Chung-Cheng Liu & Bruce M. Alberts

Department of Biochemistry and Biophysics, University of California, San Francisco, California 94143

A novel ATP-dependent DNA topoisomerase which makes reversible double-strand breaks in the DNA double helix has been purified to near homogeneity from T4 bacteriophage-infected Escherichia coli *cells. Genetic data suggest that this activity is essential for initiating T4 DNA replication forks* in vivo.

THE process of DNA replication fork movement can be successfully duplicated *in vitro* and has been shown to require several different types of purified protein[1,2]. In contrast, the process of replication intiation, whereby new replication forks are established at specific chromosome origins, has not yet been reconstituted with purified components. As a result, our knowledge of the enzymology of fork initiation is rather limited (for review see ref. 3).

Genetic studies of T4 bacteriophage development have indicated that the products of T4 genes *39*, *52* and *60* probably interact with each other to form a complex, and that this complex is essential for normal T4 DNA replication[4]. When any one of these three T4 proteins is defective, replication still begins at about the same time as in the wild type, but the rate of the initial phase of DNA synthesis is reduced[5]. This slow rate of DNA synthesis results from a low incidence of replicative forks, rather than from any reduction of the fork movement rate[6]. The products of genes *39*, *52* and *60* are thus believed to be involved in the initiation of T4 DNA replication[6].

Here, we report the discovery, properties and purification of a new ATP-dependent DNA topoisomerase. DNA topoisomerase is the name suggested for a class of enzymes that catalyse the concerted breaking and rejoining of DNA backbone bonds, as these activities are invariably detected by their ability to promote the interconversion between different topological isomers of DNA (for review, see ref. 7). The T4-induced DNA topoisomerase characterised here seems to be composed of proteins coded for by T4 genes *39*, *52* and *60*. The properties of this enzyme suggest ways in which it can act to initiate replication forks at T4 chromosome origins. A similar complex of T4 proteins has been independently isolated by Stetler, King and Huang[8], using an *in vitro* complementation assay to monitor gene *39* protein activity.

A new ATP-dependent DNA topoisomerase activity induced by T4 bacteriophage infection

Infection of *Escherichia coli* with T4 bacteriophage results in a marked increase in the ability of a cell lysate to relax covalently closed superhelical DNAs, a reaction which requires at least one transient cleavage of a DNA backbone bond[9]. This conversion of DNA circles from a negatively supercoiled form to a less supercoiled form may be sensitively detected by agarose gel electrophoresis, as shown in Fig. 1 for circular duplex PM2 DNA which has been incubated with various dilutions of an extract. Supercoil-relaxing activity is detected in this crude lysate only after extensive dilution away from the endonucleases which are also present; these nucleases convert the initial DNA substrate (form I DNA) to nicked DNA circles (form II DNA) and unit-length linear DNA molecules (form III DNA). For this reason, a group of DNA bands characteristic of partially relaxed, covalently closed DNA circles (which have an electrophoretic mobility between that of form I and form II DNAs) could be detected only after a 540-fold extract dilution. Surprisingly, no DNA supercoil relaxation was detected when the rATP in the reaction mixture was omitted. This activity was not blocked by antibody to *E. coli* ω protein or affected by the *E. coli* DNA gyrase inhibitor novobiocin (data not shown). The possibility of DNA relaxation by an excess of DNA ligase in the presence of an endonuclease was ruled out, as the same level of supercoil-relaxing activity was found in extracts of cells infected with a T4 DNA ligase (gene *30*) amber mutant (see Table 1). We therefore concluded that the activity detected in Fig. 1 originates from a previously unrecognised DNA topoisomerase. By analogy with other DNA topoisomerases, we assume that this enzyme catalyses both the breakage and the rejoining of DNA backbone bonds; moreover, it must do so in a concerted way, such that the putative intermediate in which a DNA backbone bond is broken exists only transiently. This requirement is necessitated by our failure to detect a population of nicked DNA circles as intermediates in reactions with the purified enzyme (see below).

T4 mutants of genes 39, 52 and 60 eliminate topoisomerase activity

A large number of T4 mutants were screened for the ability to affect or abolish the ATP-dependent DNA topoisomerase activity seen in Fig. 1. For this purpose, sonically disrupted cell extracts were prepared from a variety of T4 mutant-infected cells. The mutants screened for activity are listed in Table 1; of these only *am*N116 (gene 39^-), *am*H17 (gene 52^-) and *am*HL626 (gene 60^-) showed no detectable DNA topoisomerase activity in extracts. As expected, the activity was missing when these amber mutant phages were used to infect a non-suppressing (su^-) *E. coli* host (*E. coli* B); activity was present when the same mutants were used to infect an amber-suppressor strain (*E. coli* CR63). Furthermore, when any two of these three amber mutants were used to co-infect the non-suppressing *E. coli* host (*E. coli* B), the topoisomerase activity was largely recovered (see Table 1). Thus, the genetic complementation observed between mutants in these three genes *in vivo* is matched by activity complementation for the

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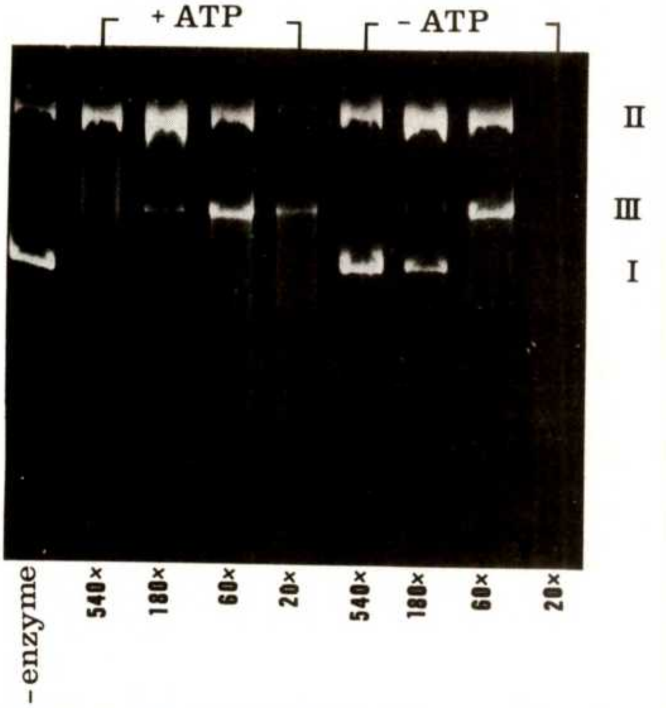

Fig. 1 An ATP-dependent DNA topoisomerase activity can be detected in extensively diluted crude extracts of T4 bacteriophage-infected *E. coli*. The Roman numerals on the right-hand margin indicate the positions after agarose gel electrophoresis of form I (covalently closed, negatively supercoiled duplex DNA circle), form II (nicked DNA circle) and form III (unit-length linear) PM2 DNA molecules. A completely relaxed closed circular DNA would co-migrate with form II DNA on this gel. The bands migrating between form II and form III DNAs in the second lane from the left (adjacent to the no enzyme control) are the covalently closed DNA circles which have a reduced superhelical density due to topoisomerase action. Note that cleavages by endogeneous nucleases produce form II and form III DNAs at high extract concentrations preventing the detection of the topoisomerase activity. Reaction mixtures (20 μl each) contained 50 mM Tris-HCl, *p*H 7.8, 40 mM KCl, 25 mM $MgCl_2$, 0.5 mM dithiothreitol, 0.5 mM Na_3EDTA, 30 μg ml^{-1} human serum albumin, 25 μg ml^{-1} covalently closed circular PM2 DNA and 0.5mM ATP (where indicated). To this mixture, 1 μl of extract diluted to various extents was added to start the reaction; the numbers below each gel lane represent the final dilution of the original extract in the 20-μl reaction. After 30 min at 30 °C, each reaction was stopped by adding 5 μl of 50% glycerol, 5% SDS and 1 mg ml^{-1} bromphenol blue and loaded on a 0.7% agarose gel. The gel was electrophoresed at room temperature in a buffer consisting of 90 mM Tris-borate (*p*H 8.3) and 5 mM $MgCl_2$ for about 15 h at 2.5 V cm^{-1}. To visualise the DNA, the gel was stained with 1 μg ml^{-1} ethidium bromide, visualised on a short-wave UV transilluminator and photographed with Polaroid film type 55. The source of infected cells for these experiments was *E. coli* strain D110 (thy^-, $polA^-$, $endI^-$), which had been infected for 30 min at 37 °C with a MOI of 10 of T4 bacteriophage [*reg*A-*am*N55(*42*$^-$)-*am*H39(*30*$^-$)] in M9 minimal medium supplemented with 0.3% casein hydrolysate, 1 μg ml^{-1} thiamine and 10 μg ml^{-1} thymidine. The infected cells (5×10^8 cells ml^{-1}) were chilled, washed and concentrated 800-fold by centrifugation. After Brij lysis[29], the lysate was centrifuged at high speed (60 min at 35,000*g*), and the supernatant was used as the extract.

topoisomerase in extracts. Note, however, that the identification of the gene 60 product as being involved here is made less clear by complications arising from *E. coli* B–*E. coli* K host-range differences (see refs 4, 5).

Because infection of *E. coli* B with T4 amber mutants in genes *39*, *52* or *60* causes a major delay in DNA synthesis, they have been designated as 'DNA-delay' (DD) mutants[10]. DNA-delay amber mutants infecting su^- *E. coli* eventually produce a substantial burst of progeny phage, although reduced relative to wild-type infection. This suggests that growth of the DNA-delay mutants on a su^- *E. coli* depends on a host function which is only partially effective[4]. Using drug inhibition studies, McCarthy recently demonstrated that one compensating host factor is *E. coli* DNA gyrase, as, unlike wild-type T4, the growth of T4 gene *39*, *52* and *60* mutants is very sensitive to the DNA gyrase inhibitors coumermycin and novobiocin[11,12]. Using autoradiography to measure the replication fork rates during gene *52* mutant infections, McCarthy *et al.*[6] have further suggested that the DNA-delay mutants are defective only in the initiation of T4 DNA replication, as the fork rates observed are normal.

Table 1 T4 mutants in genes *39*, *52* and *60* eliminate the T4 DNA topoisomerase activity

T4 mutant	Relative T4 DNA topoisomerase activity Su^- *E. coli*	Su^+ *E. coli*
Uninfected	<5	<5
*am*H39 (*30*$^-$)	(100)	(100)
*an*N116 (*39*$^-$)	<5	25
*am*H17 (*52*$^-$)	<5	25
*am*HL626 (*60*$^-$)	<5	100
*am*N116 (*39*$^-$)+*am*H17 (*52*$^-$) co-infection	20	NT
*am*H17 (*52*$^-$)+*am*HL626 (*60*$^-$) co-infection	15	NT
*am*HL626 (*60*$^-$)+*am*N116 (*39*$^-$) co-infection	30	NT

Both *E. coli* CR63 (su^+) and *E. coli* B (su^-) were grown at 37 °C in 20 ml of H broth to a cell density of 5×10^8 cells ml^{-1}; each culture was then infected at a MOI of 10, for each of the indicated T4 bacteriophages. At 12 min after infection, the cells were chilled, pelleted and resuspended in 320 μl of 50 mM Tris-HCl, *p*H 7.8, 50 mM KCl, 10 mM β-mercaptoethanol and 1 mM Na_3EDTA. Each batch of cells was broken by sonication and centrifuged for 15 min at 8,000*g* in an Eppendorf model 3200 centrifuge. Serial dilutions of each supernatant were then assayed as described in Fig. 1 legend, except that 0.2 mg ml^{-1} naladixic acid was present in each assay. The activity in each extract was estimated by comparing the relaxation activity with that in serial dilutions of the T4-*am*H39 (gene *30*$^-$, DNA ligase) infected *E. coli* B extract, whose activity was taken as 100. Other mutants tested, with activities comparable to wild type were *am*N134-*am*BL292-*am*E219 (*33*$^-$, *55*$^-$, *61*$^-$), SP62-*am*N55-*am*H39 (*reg*A$^-$, *42*$^-$, *30*$^-$), *am*HL628 (*59*$^-$), *am*N011 (*47*$^-$), *am*N130 (*46*$^-$)-*am*E727 (*49*$^-$), and the large non-essential deletions: Δ(far P13), Δ(63–32) and $\Delta(39\text{–}56)_{12}$-$alc^-$-ΔSaΔ9(ac–denB)-nd28(denA). NT, not tested.

Purified T4 DNA topoisomerase has multiple components

Starting from 100 g of T4-infected *E. coli* cells, 0.8 mg of highly purified T4 DNA topoisomerase was obtained following the multi-step procedure given in Fig. 2 legend. The total purification, estimated from specific activity measurement, was 350-fold, with a yield of 5%. With a unit of activity defined as the amount of enzyme necessary to induce half-relaxation of 0.3 μg of pBR322 DNA in 30 min at 30 °C in our standard reaction mixture (see Fig. 1 legend), the final DNA–cellulose-purified T4 DNA topoisomerase had a specific activity of 10^6 units per mg. As revealed by the Coomassie-stained SDS-polyacrylamide gel shown in Fig. 2, this fraction contains two major protein bands (molecular weights 63,000 and 52,000), which comprise about 70% of the total stained protein in the gel. Based on previous molecular weight estimates[13], these two bands are almost certainly the products of T4 genes *39* (MW assigned at 64,000) and *52* (MW assigned at 51,000), respectively. Two other protein bands with MWs of 23,000 and 16,000 are also present on our gels. Of these two species, only the 16,000-MW protein actually co-purifies with the topoisomerase activity (data not shown). In view of the data presented in Table 1, and the genetic evidence that the gene products of *39*, *52* and *60* interact with each other *in vivo*, it is tempting to identify the 16,000-MW protein as the T4 gene 60 product.

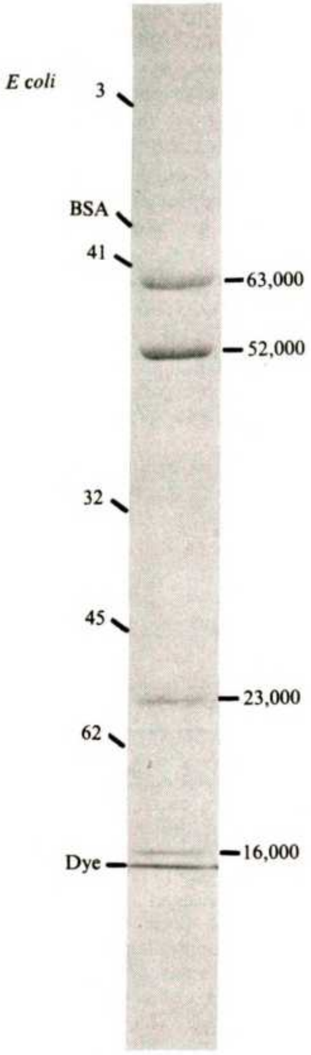

Fig. 2 SDS-polyacrylamide gel analysis of highly purified T4 DNA topoisomerase. A total of 9 μg of purified T4 DNA topoisomerase was denatured in SDS and electrophoresed on a 10% polyacrylamide slab gel[30]; the resulting protein bands were then stained with Coomassie blue as described elsewhere[31]. The approximate polypeptide MWs observed are indicated on the right hand margin, and the migration positions for other purified T4 replication proteins[29,32] used as markers (listed by T4 gene numbers), and for the *E. coli ω* protein[7], are indicated on the left hand margin. To purify the enzyme, frozen T4 (*reg*A-*am*N55-*am*H39) infected *E. coli* D110 cells (100 g) were thawed and resuspended in 150 ml of buffer containing 40 mM Tris HCl, *p*H 7.8, 2 mM Na_3EDTA, 25% sucrose and 95 mg of egg-white lysozyme (Worthington). After 60 min at 0 °C, the cells were lysed by adding 150 ml of 1% (w/v) Brij 58, 40 mM Tris-HCl and 20 mM β-mercaptoethanol and stirring at 0 °C for another 60 min. After centrifugation at 30,000*g* for 1 h, the 330 ml of supernatant was loaded on to a DEAE-cellulose (Worthington DE-52) column (2.5 cm × 24 cm) equilibrated with buffer A (40 mM Tris HCl, *p*H 7.8, 10 mM β-mercaptoethanol, 1 mM Na_3EDTA and 10% (w/v) glycerol). The flow-through fractions were pooled and precipitated by gradual addition of 10% (w/v) polymin P solution (*p*H 7.9) to a final concentration of 0.3%. The precipitate was collected by centrifugation (23,000*g* for 15 min), and the topoisomerase activity was then solubilised by extraction with 100 ml of 0.2 M NaCl (in buffer A). The topoisomerase was precipitated by ammonium sulphate (50 g per 100 ml), collected by centrifugation and redissolved in 30 ml of buffer A. After dialysis overnight against 2 l of buffer A, the topoisomerase was loaded on to a second DE-52 column (2.5 cm × 8 cm) equilibrated with buffer A. The flow through from this column was loaded directly on to a hydroxyapatite column (2.5 cm × 8 cm) equilibrated with solution B (10% glycerol, 10 mM β-mercaptoethanol) containing 20 mM potassium phosphate (*p*H 7.2). After washing the column with solution B containing 0.3 M potassium phosphate, the topoisomerase activity was eluted with solution B containing 0.5 M potassium phosphate. Pooled fractions with activity were dialysed into buffer A containing 50 mM KCl and loaded at 4 ml h^{-1} on to a DNA–cellulose column (1.2 cm × 3 cm containing 1 mg per packed ml of single-stranded calf thymus DNA). This column was eluted successively with buffer A containing 50 mM, 100 mM, 200 mM and 300 mM KCl. The topoisomerase appeared in the 200 mM KCl eluate. The peak activity was pooled and dialysed against a buffer consisting of 30 mM potassium phosphate (*p*H 7.2), 50% (w/v) glycerol, 10 mM mercaptoethanol and 0.5 mM Na_3EDTA, for storage at −20 °C, at which it seems to be quite stable.

The purified T4 DNA topoisomerase can be shown to act catalytically: one enzyme molecule (assumed to have a MW of 131,000) is capable of relaxing to completion at least seven molecules of pBR322 DNA in a 30-min incubation. The topoisomerase activity is completely resistant to novobiocin (up to 60 μg ml^{-1}), but is weakly sensitive to high concentrations of nalidixic acid. This inhibitory effect of nalidixic acid was further studied by using oxolinic acid, a more potent analogue of nalidixic acid; the T4 DNA topoisomerase activity was halved by oxolinic acid at about 250 μg ml^{-1}. As this level of oxolinic acid is about an order of magnitude higher than the concentrations needed for the inhibition of *E. coli* DNA gyrase[14,15], it is likely that the observed inhibition is nonspecific. A similar 'nonspecific' inhibition has been previously observed for another DNA topoisomerase, *E. coli ω* protein, at comparably high oxolinic acid concentrations (500 μg ml^{-1}, ref. 15). When tested with a variety of different circular DNA molecules, the topoisomerase activity shows no obvious DNA preference. The purified enzyme exhibits no DNA gyrase activity with either fully relaxed pBR322 DNA or PM2 DNA in our assay conditions (that is, it is unable to introduce supercoils into these DNA molecules).

Both in the crude extract and in the first DEAE-cellulose breakthrough, the topoisomerase activity is associated with a 30S complex, as judged by sucrose gradient sedimentation (data not shown). This complex can be destroyed, without affecting the ATP-dependent relaxation activity, either by pancreatic RNase A treatment or by salt-extracting the enzyme from a precipitate formed with the synthetic polycation polymin P, as described in Fig. 2 legend. The significance of this apparent RNA association in crude fractions is not known. However, our purified enzyme contains less than 1% nucleic acid, and it sediments more slowly than 5S.

The topoisomerase can catalyse the ATP-dependent relaxation of positive DNA twists

Unlike the *E. coli ω* protein (which can only remove negative turns), T4 DNA topoisomerase catalyses the relaxation of both negatively and positively superhelical DNAs. To assay for the relaxation of positively supercoiled DNA, a fully relaxed PM2 DNA substrate was prepared, and ethidium bromide was added to make this relaxed DNA act as if positively supercoiled. The relaxation of the positive DNA twists present in these conditions can be detected by the increased mobility of the covalently closed PM2 DNA, seen when this DNA is subjected to electrophoresis in the absence of ethidium bromide (see Fig. 3*a*). With our highly purified T4 DNA topoisomerase, we have also demonstrated that: (1) the relaxation of both negative and positive twists is ATP dependent; (2) both reactions are gradual, indicating that the enzyme does not relax the DNA by a one-hit mechanism and that it dissociates from each DNA molecule before relaxing it completely, (3) the enzyme acts catalytically in both cases, in that one T4 DNA topoisomerase molecule can relax many PM2 DNA molecules in a 30-min reaction (data not shown).

ATP hydrolysis is required for catalytic relaxation

The relaxation of superhelical DNAs of both senses is a thermodynamically favourable process. Correspondingly, none of the relaxation reactions catalysed by the other known DNA topoisomerases requires an exogenous source of energy[7]. In contrast, we have shown (Fig. 1) that the T4-induced DNA topoisomerase requires ATP as a cofactor. The requirement for ATP cannot be replaced by other ribo- or deoxyribonucleoside

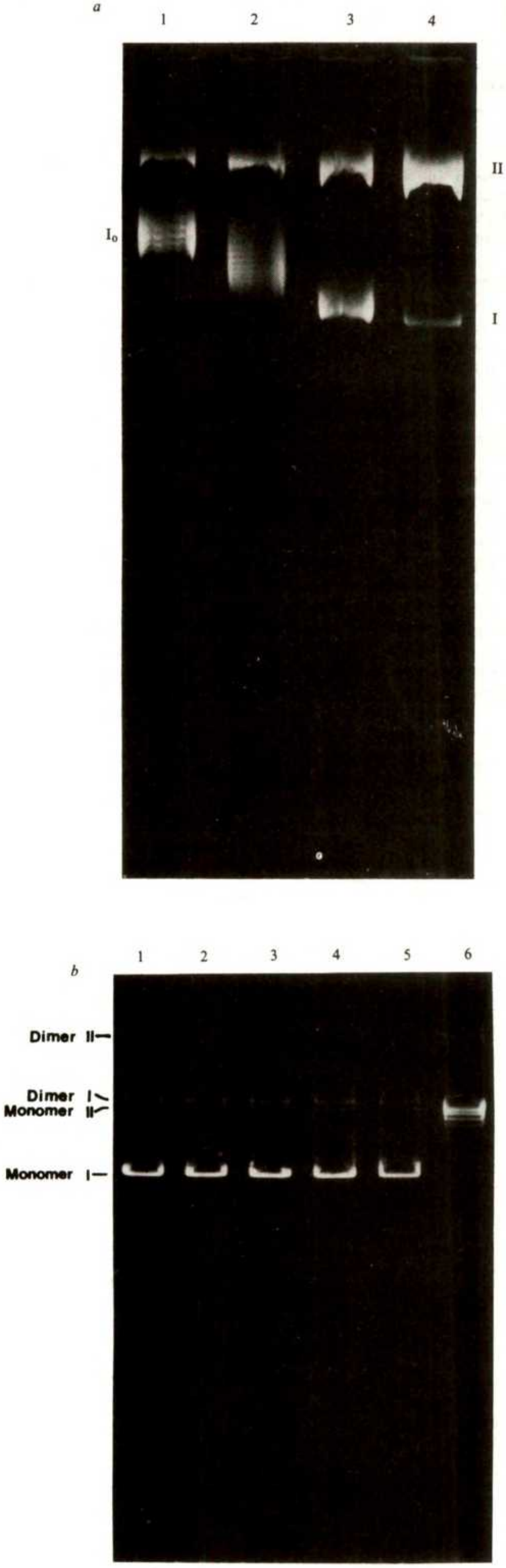

Fig. 3 *a*, The ATP-dependent relaxation of positively superhelical PM2 DNA catalysed by the T4 DNA topoisomerase. Reactions were carried out as described for Fig. 1, except that fully relaxed, covalently closed PM2 DNA (form I_0) was the substrate and ethidium bromide was present at 2 $\mu g\ ml^{-1}$ to induce positive superhelical turns[33]. As the source of the topoisomerase, the breakthrough of the initial DE-52 chromatography step (described in Fig. 2 legend) was used. This partially purified topoisomerase was diluted into the reaction mixture as follows: lane 1, no enzyme; lane 2, 2,000-fold; lane 3, 500-fold; lane 4, 100-fold. Electrophoresis was carried out as described for Fig. 1, but at 4 °C. The removal of ethidium bromide during electrophoresis causes the unreacted substrate DNA to return to a relaxed conformation, but a small amount of negative superhelicity is induced by the low temperature used for electrophoresis. As a result, at 4 °C, the substrate DNA (denoted as form I_0) migrates faster than nicked circular DNA (form II), but more slowly than the highly negatively supercoiled form I DNA. In contrast, the DNA relaxed in the presence of ethidium bromide gains a great deal of negative superhelicity on removal of this dye; thus, the final product of the topoisomerase reaction is the rapidly migrating form I DNA. Note that, with increasing enzyme, increasing numbers of negative supercoils are induced by the topoisomerase in all the covalently closed DNA circles which survive the incubation; this change in superhelicity is only obtained when ATP is present (data not shown). In addition, the topoisomerase still contains an endonuclease contaminant at this stage, which causes the amount of form II DNA to increase as more enzyme is added. *b*, When inducing DNA relaxation, the topoisomerase acts catalytically and requires ATP hydrolysis. Reaction mixtures (20 μl each) contained 50 mM Tris-HCl, *p*H 7.8, 60 mM KCl, 10 mM $MgCl_2$, 0.5 mM dithiothreitol, 0.5 mM Na_3EDTA, 30 $\mu g\ ml^{-1}$ human serum albumin, 20 $\mu g\ ml^{-1}$ form I pBR322 DNA (negatively supercoiled) and 0.2 $\mu g\ ml^{-1}$ of the most highly purified fraction of T4 DNA topoisomerase. Incubations were carried out for 30 min at 30 °C, and the products were analysed as described for Fig. 1. Lane 1 is the no enzyme control; lanes 2–6 represent reactions carried out with 70 μM rATP present, plus different amounts of the non-hydrolysable ATP analogue, ATP-γS, as follows: lane 2, 500 μM; lane 3, 200 μM; lane 4, 50 μM; lane 5, 10 μM; lane 6, no ATP-γS. As nearly complete inhibition of the topoisomerase activity occurs with 10 μM ATP-γS in the presence of 70 μM ATP (lane 5), it is clear that the K_i for ATP-γS is much lower than the K_m for ATP.

triphosphates. This surprising energy requirement has prompted us to investigate the ATP dependence further.

The fact that actual ATP hydrolysis (rather than merely the presence of ATP) is required comes from two lines of evidence. First, the compound rATP-γS [riboadenosine 5′-*O*-(3-thiotriphosphate)] inhibits the topoisomerase reaction strongly when added to our normal reaction mix, as shown in Fig. 3*b*. rATP-γS is an analogue of rATP which is poorly hydrolysed by enzymes which cleave the β–γ phosphodiester linkage of ATP. It acts as an inhibitor of many ATPases, including the activities of the T4 gene *41* and *44/62* proteins (unpublished observation of this laboratory). Second, as shown in Table 2, a DNA-dependent ATPase activity can be detected in the most purified fraction of T4 DNA topoisomerase, for which the hydrolysis products are ADP and inorganic phosphate. With certain assumptions (see Table 2 legend), we can calculate that the number of ATP molecules hydrolysed is roughly equal to the number of superhelical DNA turns relaxed by the topoisomerase.

It is important to note that although rATP-γS is a potent inhibitor of the T4 DNA topoisomerase-catalysed relaxation reaction, it can, nonetheless, stimulate the relaxation reaction in the absence of ATP when the enzyme is present at substrate levels (C.-C.L., unpublished results). This situation is analogou to the effect of a non-hydrolysable analogue of A App(NH)p, on *E. coli* DNA gyrase[16]. For gyrase, the bind ATP seems to induce a conformational change in the which leads to a single cycle of DNA supercoiling; the of ATP is then required to return the enzyme to conformation[16]. The situation for the T4 DNA to probably similar, except that one cycle of D

Table 2 DNA-dependent ATPase activity of the T4 DNA topoisomerase

	ATP hydrolysed (nmol ml^{-1})		
	5 min	10 min	15 min
−DNA	<0.2	< 0.2	0.2
+35 μg ml^{-1} ss fd DNA	0.9	1.9	2.5
+20 μg ml^{-1} pBR322 DNA	2.0	4.0	5.0
+50 μg ml^{-1} T4 ds DNA	3.7	8.3	10.5

The reaction mixtures (20 μl each) were as described in Fig. 1 legend, except that 100 μM [γ-^{32}P]ATP was present. The most purified T4 DNA topoisomerase (0.5 μg) was added to each reaction mixture. After incubation at 30 °C for the times indicated, aliquots (50 μl) of reaction mixtures were withdrawn and added to 0.5 ml of 0.1 M HCl. Hydrolysis was measured as inorganic ^{32}P (as phosphate) separated from unhydrolysed [γ-^{32}P]ATP by charcoal adsorption[27]. The different DNAs present in each reaction mixture are indicated. That the products of hydrolysis are ADP and inorganic phosphate was determined in a separate experiment in which ^{3}H-ATP was used as substrate and the products were analysed by chromatography on PEI-cellulose[28]. From the ATP hydrolysis observed during a 30-min incubation at 30 °C using 0.5 μg of enzyme and 100 μM ATP, we calculate that about 1 pmol of ATP would be hydrolysed by 2.5 ng of enzyme (assuming that the dose response is linear for ATPase activity). In the same conditions, 2.5 ng of enzyme will relax about half of the superhelical turns in 0.1 pmol (as DNA molecules) of pBR322 DNA. As there are about 30 superhelical turns in each pBR322 DNA molecule in our reaction conditions, we estimate that one or two superhelical DNA turns are relaxed for every ATP molecule hydrolysed.

rather than DNA supercoiling, is observed on ATP binding. However, we cannot rule out the possibility that the stimulation is due to a very slow hydrolysis of ATP-γS by the topoisomerase.

Similarities between the T4 DNA topoisomerase and *E. coli* DNA gyrase

At least two kinds of DNA topoisomerase are found in bacteria, as exemplified by the *E. coli* ω protein (*Eco* DNA topoisomerase I) and the *E. coli* DNA gyrase (*Eco* DNA topoisomerase II). *E. coli* ω protein catalyses the energetically favourable partial relaxation of negatively superhelical DNA with no added energy cofactor[17]. In contrast, DNA gyrase[36] catalyses the reverse reaction, introducing negative superhelicity into DNA in a reaction requiring ATP hydrolysis[16,18].

The T4 topoisomerase and the *E. coli* gyrase are multiple-subunit proteins which have both nicking–closing and DNA-dependent ATPase activities. In both cases, these two activities are apparently tightly linked, inasmuch as each cycle of nicking and closing needs to be accompanied by hydrolysis of one (or two) ATP molecules[16,18]. Finally, for both enzymes addition of SDS and protease seems to trap nicked DNA–enzyme intermediates, in which both DNA strands have been broken to produce a unit-length linear DNA molecule from a double-stranded DNA circle (refs 14, 15 and L.F.L., unpublished results). Nevertheless, there is at least one important difference: only the *E. coli* gyrase is able to harness its ATP hydrolysis energy to do useful work (induce DNA supercoiling); so far in our experiments the T4 DNA topoisomerase seems to be wasting this energy, as the reaction catalysed is already thermodynamically favourable without ATP hydrolysis.

might T4 DNA topoisomerase function ork initiation?

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initiation, by formation of an *in* *vivo*-like 'replication bubble' on a double-stranded T4 DNA molecule, has not been achieved in this (or any other) purified *in vitro* system. The previously described genetic evidence implying that the T4 gene *52*, *39* and *60* products are specifically involved in T4 replication fork initiation, coupled with the present identification of these proteins as a topoisomerase, suggests that this enzyme may be the missing factor.

The tight coupling between the ATP hydrolysis and relaxation activities of T4 DNA topoisomerase is unique among all known DNA topoisomerases. Although it is possible that the ATP hydrolysis functions directly in the nicking–closing reaction (for example, by being used to provide energy for ligation of the putative broken DNA chain intermediates), it seems much more likely that the ATP hydrolysis energy is normally coupled to another, as yet unidentified, energy-utilising reaction.

We have no direct evidence as to how ATP energy is used by the topoisomerase at the replication origin. However, we will present a speculative model which is suggested by the above-mentioned similarities between the T4 DNA topoisomerase and DNA gyrase. In this view, the T4 DNA topoisomerase serves as a DNA gyrase-like enzyme which acts specifically at the T4 replication origin. Such an enzyme might function to initiate new T4 replication forks in a manner analogous to that schematically outlined in Fig. 4. Here, the T4 DNA topoisomerase would recognise two specific DNA sequences spanning the origin[20] of T4 DNA replication. By anchoring these two DNA sequences on the same enzyme molecule and thereby restricting the rotation of the DNA strands relative to the enzyme, the topoisomerase could create a closed topological domain. This would allow its hypothetical ATP-driven gyration to induce a local negative supercoiling which destabilises the double helix at the replication origin. As indicated, the melting of this origin would presumably require the gene *32* helix-destabilising protein. Participation of the T4 gene *41* and/or gene *44*/*62* replication proteins, destabilising the helix by ATP-driven walking

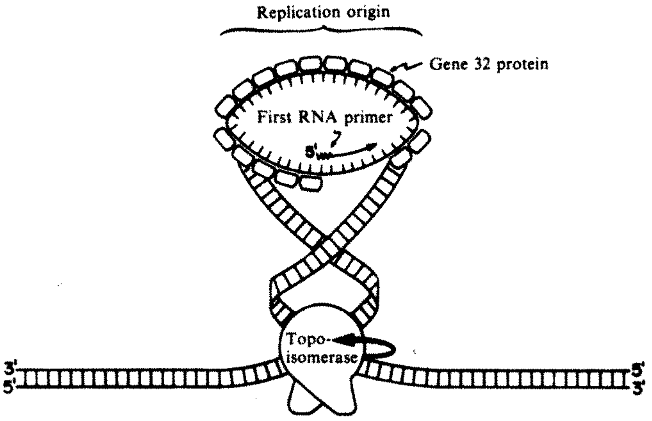

Fig. 4 Two possible models for T4 DNA topoisomerase action at the T4 replication origin. These two models assume (without proof) that the rate-limiting step in generating new replication forks is the opening of the DNA helix at the origin (see text). *a*, Topoisomerase gyration. In this hypothetical scheme, the topoisomerase acts as a site-specific DNA gyrase and uses its ATP hydrolysis and nicking–closing activities to seperate the strands of the DNA helix at the T4 replication origin. The replication origin must form a closed loop, as an isolated domain is required in the otherwise linear T4 DNA molecule to allow supercoiling. This loop could be formed either by the binding of the topoisomerase alone (as indicated here) or by a separate set of proteins or membrane sites. The T4 gene 39 and 52 proteins have been reported to interact with the cell membrane, as well as with DNA[34], and so some membrane attachment might also be used to constrain the DNA origin in this way. (It is interesting that limited topological domains have been detected in the condensed T4 chromosome[35].) In any case, the topoisomerase is suggested to recognise a set of specific DNA sequences which designate a T4 chromosome origin, and to show a DNA gyrase-like activity there (induction of negative twists which destabilise the helix) which is not detected with the non-origin DNA sequences present in our standard topoisomerase assay.

along DNA single-strands[21,22], is also possible. Note that, in this view, the ATP-dependent relaxation of non-origin-containing DNA molecules observed *in vitro* reflects an uncoupled version of a site-specific DNA gyration reaction catalysed by the topoisomerase *in vivo*.

The site-specific gyration model in Fig. 4 postulates that the initial opening of the DNA helix is followed by an RNA-primed DNA chain start, which actually begins DNA synthesis. This first RNA primer could either be synthesised by the same enzymes that prime the Okazaki pieces made on the lagging strand of the T4 replication fork, or be made by a separate mechanism[3,23]. It has been shown recently that the T4 gene *61* protein, in conjunction with the T4 gene *41* protein, can synthesise pentaribonucleotides, and that this synthesis has an absolute requirement for a single-stranded DNA template (ref. 19 and C.-C.L., unpublished results). When additional replication proteins are present, these short ribonucleotides will prime lagging strand DNA synthesis in the T4 *in vitro* system[19]. These two T4 proteins may therefore also have a crucial role in replication fork initiation.

Despite our limited knowledge concerning the mechanism of initiation of DNA replication forks, some similarities can be seen between different systems. As is the case for many other genomes (bacteriophage λ, *E. coli* and eukaryotic chromosomes), replication forks in T4 seem to be able to start out bi-directionally from an initial replication bubble[24]. We therefore expect that a detailed study of the T4 DNA topoisomerase and its interaction with the other T4 replication proteins can provide a model system in which some general properties of fork initiation mechanisms can be elucidated.

Genetic data suggest that genes *O* and *P* of bacteriophage λ may act in a manner analogous to T4 DNA topoisomerase. As for T4 genes *39*, *52* and *60*, the λ *O* and *P* products interact with each other, and when their levels are decreased, the initiation of λ DNA replication is altered[25]. Experiments comparing various lambdoid phages have shown the gene *O* product is highly phage specific, presumably because it recognises origin DNA sequences[26]. Similarly, unlike most T4 genes, gene *39* function cannot be complemented by bacteriophage T2 (W. M. Huang, personal communication); this suggests that gene *39* may have a role in T4 replication which is analogous to the role of gene *O* in λ replication.

Further study of the T4 DNA topoisomerase should also provide valuable knowledge concerning the functions and mechanisms of the important class of enzymes known as DNA topoisomerases. In this respect, we have observed that, at much higher enzyme concentrations (about equal weights of DNA and T4 DNA topoisomerase), a novel ATP-independent DNA topoisomerisation reaction can be detected, in which topologically knotted, double-stranded superhelical DNA molecules are created. Most strikingly, these knots can subsequently be removed by catalytic amounts of the topoisomerase, in an efficient ATP-dependent reaction. As will be described in detail elsewhere, such data reveal that the T4 DNA topoisomerase is working by making transient double-stranded DNA breaks which are rapidly and efficiently resealed in its 'closing' reaction.

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Erratum

In the letter 'Basaltic pillars in collapsed lava-pool on the deep ocean floor' by J. Francheteau *et al.*, *Nature* **281,** 209–211, the third author's name was mis-spelt; it should read C. Rangin. Figures 1 and 2 were incorrectly reproduced. They are shown correctly below.

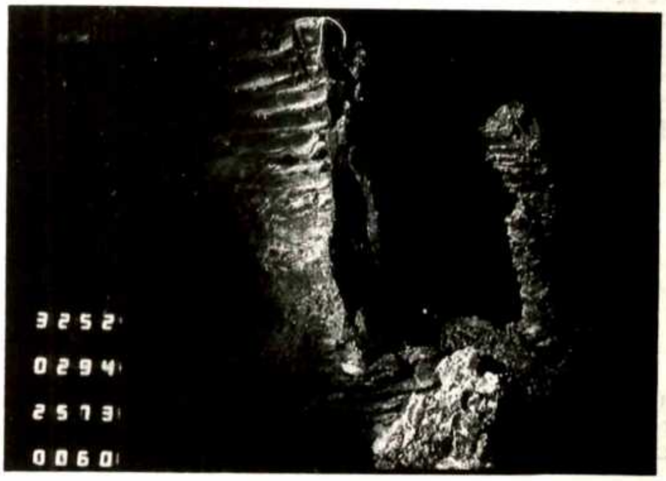

Fig. 1 General landscape: pillars with collapsed lava pond.

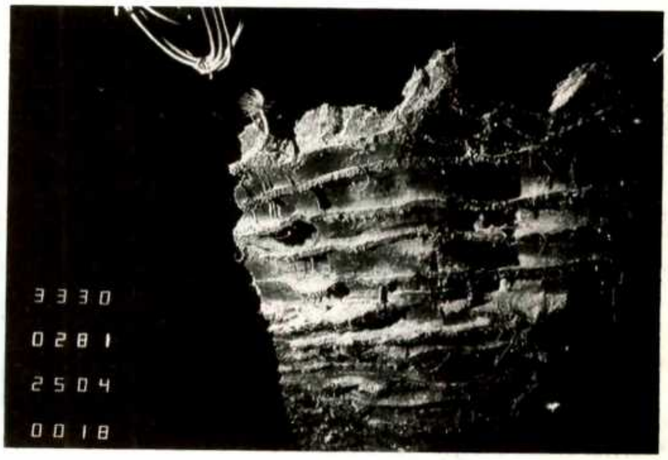

Fig. 2 Detail of a pillar showing the centimetric pseudo layering with thin and darker salient glass layers projecting from the basaltic surface of the pillar.

letters

HEAO 1 observations of 4U1232+07

S. P. Bhavsar & M. P. Ulmer

Department of Physics and Astronomy, Northwestern University, Evanston, Illinois 60201

R. G. Cruddace, K. Wood, J. Meekins, D. Yentis, W. Evans, H. Smathers, E. T. Byram, T. A. Chubb & H. Friedman

E. O. Hulburt Center for Space Research, Naval Research Laboratory Washington, D.C. 20375

While performing an analysis of the NRL HEAO 1 observations of the Virgo cluster and 3C273, we detected X-ray emission from a region between these two objects. The most satisfactory explanation is the presence of two weak X-ray sources, for which we have obtained error boxes. One source is likely to be the peculiar galaxy IC3576, suggested by Margon *et al.*[1] as the source 2U1231+07 = 4U1232+07 (ref. 2). The error box resulting from combining our observations with the 4U data strengthen the X-ray identification with this extremely interesting galaxy. There are numerous possibilities for the other X-ray source, and here we discuss one, the interacting pair of galaxies NGC4410a and NGC4410b (NGC4410a/b).

The field of view of the X-ray detectors is 4° × 1°. The data we used were obtained from the superimposition of parallel scans within ±0.75° of the arc defined by α = 12 h 50.5 min, δ = 16.2° to α = 12 h 17.5 min, $\delta = -1.6°$. The relationship of the 4° × 1° field of view to the scan, and the sky position of several objects is shown in Fig. 1. The average number of counts per 0.5° bin are plotted in Fig. 2. The data have been fitted to a diffuse source model for the Virgo cluster and two point sources for the region between 348 and 352 deg. The 3C273 data were not included in these fits. The least squares fit centres the Virgo cluster at scan angle = 5.9° and the two point sources at scan angles 9.3° and 10.5°, as shown in Fig. 2. The region between 9 and 13 deg is fitted equally well by a diffuse source of core radius 176 arc min, but it is unlikely that a diffuse source so large would be in this region. This region is ≳7° from the Virgo Cluster centre or M87, and, hence, is unlikely to be an extended region of hot gas within the Virgo Cluster. Also, there are no rich clusters or superclusters in this direction, and therefore we believe two point sources to be a more likely interpretation of the data.

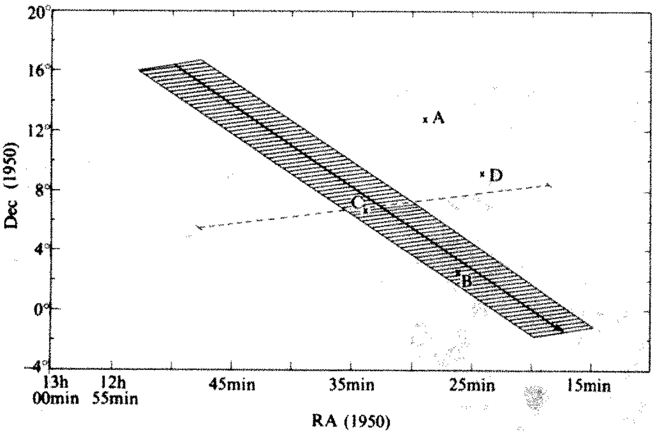

Fig. 1 The ±0.75° wide shaded region shows the portion of the sky covered by the centre of the field of the X-ray collimator over several scans. The arrow shows the direction of the scans for reference with Fig. 2. The dashed line (±4°) delineates the 4° direction of the X-ray collimator (see text). The positions of A, B, C and D are for M87, 3C273, IC3576 and NGC4410a/b, respectively.

The HEAO and Uhuru error boxes are shown in Fig. 3. The corners of the parallelogram lying in both boxes are given by: α = 12 h 35 min 30 s, δ = 6°50′; α = 12 h 31 min 10 s, δ = 7° 19′; α = 12 h 35 min 07 s, δ = 6°38′; α = 12 h 30 min 36 s, δ = 7°10′; and the centroid of the error box is at α = 12 h 33 min 06 s, δ = 6°59.2′. The coordinates of IC3576 are α = 12 h 34 min 5.4 s and δ = 6°53′47″. The area of the parallelogram is 0.25 deg^2. We conclude that the identification suggested by Margon *et al.*[1] is correct. IC3576 is listed as a dwarf galaxy by van den Berg[4], but this is doubtful (see Margon *et al.*[1]). It has been studied in detail at optical and radio[1] wavelengths, and we summarise these results as follows: (1) its magnitude is 15.2 (ref. 5). The galaxy has no well-defined spiral structure and has several blue condensations which might be OB associations; (2) radio observations of the galaxy show a very unusual ratio of neutral hydrogen to optical luminosity, the ratio being almost 10 times greater than that for a normal galaxy and almost twice that for a dwarf galaxy. Its distance is about 20 Mpc[1], and therefore its X-ray luminosity (1–10 keV) is 10^{42} erg s^{-1}. This is about 500 times stronger than that for our own galaxy. IC3576 is clearly worth further investigation.

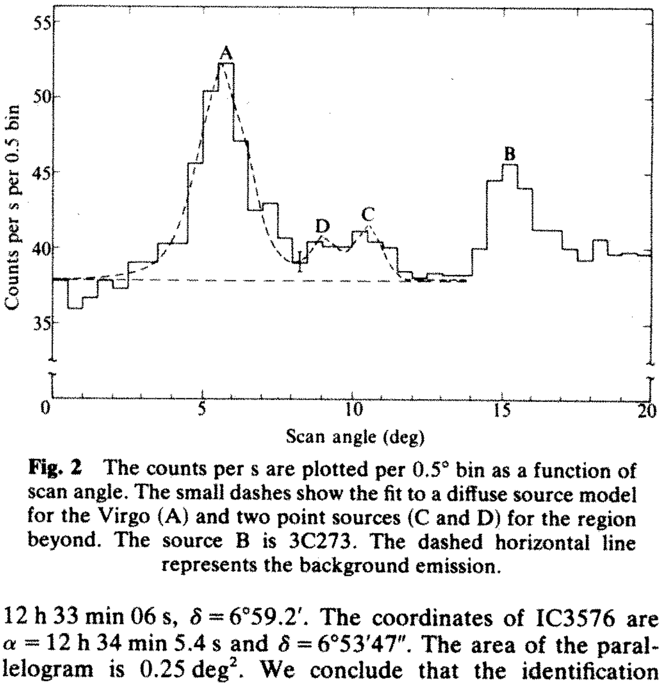

Fig. 2 The counts per s are plotted per 0.5° bin as a function of scan angle. The small dashes show the fit to a diffuse source model for the Virgo (A) and two point sources (C and D) for the region beyond. The source B is 3C273. The dashed horizontal line represents the background emission.

The centroid for the other X-ray object is α = 12 h 34 min 57.6 s, δ = 8°11′24″. The corners of the 90% confidence error box are: α = 12 h 46 min 38.4 s, δ = 7°18′36″; α = 12 h 45 min 12 s, δ = 6°32′24″; α = 12 h 24 min 36 s, δ = 9°49′12″; α = 12 h 23 min 9.6 s, δ = 9°2′24″.

We found no QSOs (in the list by Burbidge *et al.*[6]) in the error box. There is one 4C object, 4C09.41 (ref. 7), just outside the error box, and the interacting pair of galaxies NGC4410a/b within the error box. Condon and Dressel[8] have suggested that a

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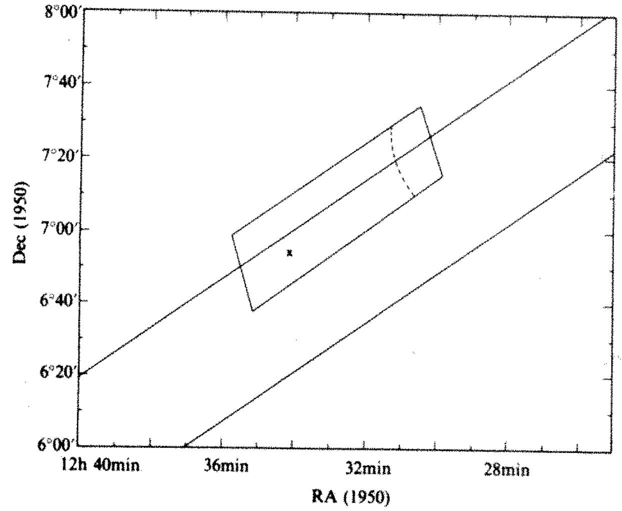

Fig. 3 The error box for 4U1232 + 07 is shown and superimposed on it is a portion of our error box (the two parallel lines). The dashed line, combined with the left hand portion (east) of the 4U error box is the allowed region for the source determined by Margon *et al.*[1]. × represents the position of IC3576 on the sky.

quiescent galactic nucleus might be triggered into radioactivity by a close encounter between two galaxies, and Griffiths *et al.*[9] have extended this idea to the X-ray range by suggesting that interacting pairs are likely to be X-ray sources. Schanberg[10] has shown that the pair NGC4410a/b is indeed interacting, so that, for example, tidal distortion is evident in the outer regions of NGC4410a/b. If the system is an X-ray source, its luminosity is about 5×10^{43} erg s^{-1}.

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Near IR variability of the quiet Sun

M. N. Markov, B. V. Volynov, V. M. Zholobov, V. V. Gorbatko, Ju. N. Glazkov, V. S. Petrov, Ju. S. Ivanov & N. O. Dombrovsky

P. N. Lebedev Physical Institute, Moscow, USSR

The IR region of the solar spectrum provides important diagnostics for heat transfer processes in the photosphere, where the gravity and acoustic waves which heat the corona originate. Rates of molecule formation and dissociation in these layers are highly temperature sensitive. Recently relative intensities and short-period variations in individual lines of the rotation–vibration band of CO at 4.7 μm (refs 1–3) were obtained with spectral resolution $\nu/\Delta\nu \sim 0.7$–2.1×10^4 (refs. 4, 5) from ground-based sites. High resolution cannot wholly overcome atmospheric absorption problems, so to measure absolute energy and variability in the band we have observed the solar spectrum from the orbital station Salyut 5.

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The spectrometer for the region 1.8–15 μm used a bolometric detector and a Littrow–Pfund optical layout with an NaCl prism. It was fed by a newtonian telescope of 45 cm focal length with a 19 cm × 24 cm primary, giving a field of view of 4 arc min × 24 arc min as defined by the entrance slit. A typical recording time per spectrum is 4 s. The station served as an inertial observing platform, pointing to within an accuracy of a few arc min; stability was maintained via a solar imaging camera, with 2.5 s between images.

Of the spectra taken on 1, 4 and 5 August 1976 and 16 February 1977 we have chosen 46 from the east–south part of the disk, where independent data[6] showed no solar activity such as spots or flares. The situation is illustrated in Fig. 1. Table 1 shows the timing of the selected groups of spectra.

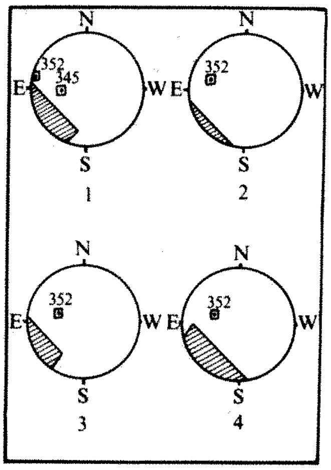

Fig. 1 The hatched areas show the regions on the solar disk on which the four groups of experiments were directed. ⊡, The regions of solar activity during the experiments.

Figure 2 shows the results of averaging each group of spectra in the wavelength range 4–6 μm where variability was observed. Between 4.05 and 5.25 μm there are excesses: for series 3 of 3%, for series 2 of 8% and for series 1 of 13% compared with the minimum curve of series 4. The r.m.s. error in the data is between <1% and 2%. An estimate of systematic error was obtained from data with the slit 3 arc min from the solar limb, when a signal value of 1% was registered. Guidance errors are 1 arc min maximum, and scattered light was minimised by diaphragms at three pupils: the spectrometer slit, the output slit and the bolometer. We can thus assert that the observed CO

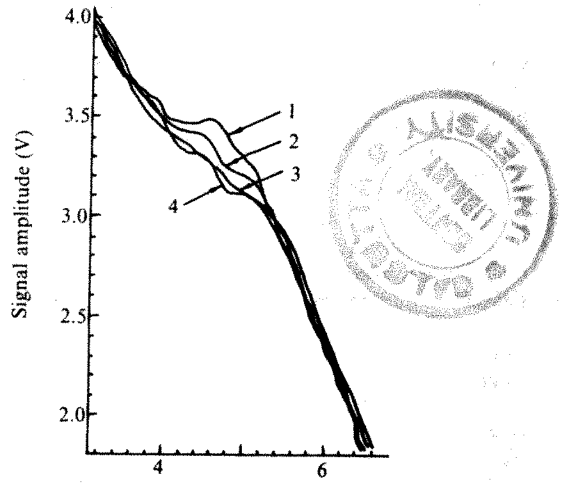

Fig. 2 Spectral dependence of a signal at the spectrometer output for the experimental groups 1, 2, 3 and 4.

Table 1 Timing of the 46 selected spectra

Series	Orbit no.	No. of spectra	Date (August 1976)	Time duration	$F_{\odot}$(2,800 MHz) (Wm^{-2} Hz^{-1})
1	640	24	1	11 h 21 min 40 s–11 h 23 min 20 s	75.9×10^{-22}
2	685	6	4	7 36 35 – 7 37 0	82.5×10^{-22}
3	700	6	4	6 43 50 – 6 44 15	82.5×10^{-22}
4	701	10	5	7 23 45 – 7 33 25	84.2×10^{-22}

Timing relates to the spacecraft orbit, and thus bears no periodic relation to the period of solar rotation or activity.

variability is real, and intrinsic to the quiet Sun. Absence of solar activity is indicated by the steady values of the 2,800 MHz radio flux $F_{\odot}$ (2,800 MHz) shown in Table 1. Geomagnetic field perturbations were low (index $A_p \sim 4$–5) and two small Hα flares, shown as 352 and 345 in Fig. 1 do not lie near our observing regions. OSO 8 X-ray data and Mt Wilson magnetograms listed in ref. 6 also indicated no flare activity there.

From previous, resolved spectra of the solar CO band[4,5] one can conclude that the species contributing to the radiative flux are H^- (at an effective temperature of 5,400 K (ref. 7)) and CO (with $T \sim 4{,}500$–4,900 K). The linewidth to separation ratio for CO is 1:10 so the CO absorption cannot be >10% of the continuum in the range 4.05–5.25 μm. An observed variability of 5–10% suggests complete disappearance and re-formation of CO over periods of the order of a few hours. This could be due to convective cycling, in the granules, of hotter material from below.

The observed variability in flux is of the order of 1.3×10^4 W m^{-2}, that is, some 2×10^{-4} of the total flux of solar radiation. Theoretical estimates for the energy flux contained in gravity waves lie close to 4×10^4 W m^{-2} with an acoustic flux of similar order. Thus CO formation and destruction may provide an important sink and source of energy for the maintenance of the acoustic, gravity, and magneto-acoustic waves responsible for coronal heating. Although details must await a more thorough analysis, one can point to suggestive theoretical support for the hypothesis. Theoretical values[8] for the non-radiative component of the energy flux are much greater than the combined fluxes due to X-ray and UV radiation and the solar wind; CO radiation could balance the energy equation. Photospheric thermal relaxation could be affected by the CO radiation, thus affecting the propagation of gravity waves. Concentration changes in CO can enhance photospheric convective instability by changing the radiative temperature gradient. Finally, note that further observations with greatly increased angular resolution could be used to make direct observations relating to the energetics of convective granulation.

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An estimate of the fine structure constant

S. L. Glashow & D. V. Nanopoulos

Lyman Laboratory of Physics,
Harvard University, Cambridge, Massachusetts 02138

Quantum electrodynamics, despite its many successes, has left many questions unanswered. Why is electrodynamics so different from other interactions? Why do all particles have commensurate electric charges? Why is the value of the fine-structure constant what it is? Today, we have an apparently correct and essentially complete description of all of elementary particle physics in terms of a gauge theory[1]. If the underlying gauge group is simple[2,3], all three elementary particle interactions—strong, weak, and electromagnetic—are parts of one unified theory. (The earlier attempt to synthesise all three interactions obtained both charge quantisation and baryon number violation. It was not based on a simple gauge group, nor did it incorporate conventional quantum chromodynamics.) Electromagnetism is no longer so unique. Apparent differences among the interactions reflect the pattern of spontaneous symmetry breakdown. Charge quantisation is forced on us: all fields have integer mutiples of one-third the electron's charge[2,3]; all observable particles have integer multiples. (We assume that colour is a truly hidden variable, that quarks are fractionally charged and never liberated. For another point of view, see ref. 4.) The fine-structure constant cannot be chosen arbitrarily. For unified theories in which the proton is unstable, we find an upper limit to its strength, $\alpha < 0.04$. We derive this result here and survey the context in which it arises.

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The minimal gauge group which is required to describe all three interactions is $H = SU(3)\times SU(2)\times U(1)$, not a simple group. Three kinds of quantum fields are needed: gauge bosons, Higgs bosons, and fermions: the 12 gauge bosons include eight gluons (confined, hence not directly observable), three intermediate vector bosons (heavy, hence not yet observed), and the photon. At least one Higgs boson must survive as an observable particle at accessible mass[5,6].

Fermions come in families of 15 chiral fields apiece[7]. (We put the tau lepton and the upsilon-constituent into a third 15-plet, also including a not-yet-seen $Q = 2/3$ quark.) One family—containing up and down quarks, electrons, and electron neutrinos—suffices for a description of most phenomena. There exist at least two additional families. We do not know why all families have the same format, nor why there are three families, nor whether there are more than three. (The $SU(3)\times SU(2)\times U(1)$ representation content of each of the families is identical. Each family is a minimal anomaly-free representation of H. This behaviour survives unification in its simpler forms. Each family is a reducible $10+\bar{5}$ representation of SU(5), or an irreducible spin representation of 0(10). In no plausible scheme do all three families belong to a single irreducible representation.) Each family is supposed to contain a weakly-coupled massless, or nearly massless, neutrino. The number of these that may exist is related to the observed helium abundance in the Universe[8–10], and not many more than three families are tolerable. On the other hand, three is the minimum number of families for which an elegant description of CP violation makes sense[11].

The theory based on the gauge group H and involving three fermion families is not a unified theory. It does not explain charge quantisation. It involves 17 independent dimensionless parameters[12], each as fundamental as the fine-structure constant. Although 17 free parameters must be too many for a fundamental theory to admit, it should be considered that all elementary particle properties can be expressed (in principle) in terms of them. Perhaps the gauge group H is merely the phenomenologically-relevant remnant of a larger and simple unifying group G. The three independent gauge coupling constants are reduced to one, and quantisation of charge is assured. Restrictive hypotheses can yield further relationships among the 17 parameters[13]. Such theories have the following properties: (1) G has more generators than does H. They correspond to a fourth class of gauge boson which mediates new types of interaction. Generally, the new interactions leads to the decay of the proton. (But not necessarily. Unified theories without proton decay exist. A scheme based on $[SU(3)]^3$ with permutation symmetry is an example.) These gauge bosons must therefore be extraordinarily heavy. (2) The value of the weak mixing angle $\sin^2\theta$ can be computed, and the result agrees with experimental determinations.[14] This is the only convincing quantitative indication that unification is correct. (3) An interplay between CP violation and baryon number violation can, during the very early history of the Universe, produce the observed baryon surplus.[15-19] This result requires the existence of a rich structure of Higgs bosons,[20,21] a fact which is confirmed by the observed pattern of fermion masses. For unified schemes based on either SU(5) or O(10), the Higgs bosons which couple to fermions must comprise at least two inequivalent representations. For SU(5), at least a 5-plet and a 45-plet are needed. For O(10), at least a 10-plet and a 126-plet are needed. In either case, the Higgs sector is then rich enough to provide the baryon surplus.

The superheavy unification mass may be estimated in two ways. Strong interactions are asymptotically free, so that the strong gauge coupling constant decreases with increasing energy. The fine-structure constant slowly increases. The point of convergence of the coupling constants dictates the unification mass. We obtain $\sim 10^{14}$ GeV assuming that we have correctly identified H as the entire phenomenological gauge group[22,23]. The existence of baryon-number violating interactions must soon reveal itself.

An alternative estimate of the unification mass does not depend on the details of particle phenomenology. It follows from the present lower limit on the proton lifetime of 10^{29} yr (ref. 24). This assures us that the mass M of the baryon-number violating intermediary must be $\geqslant 10^{14}$ GeV. It also provides our upper limit on the strength of the fine-structure constant. We have noted that electromagnetism is not asymptotically free. The fine-structure constant depends on energy according to[25,26]

$$\alpha^{-1}(0) = \alpha^{-1}(M^2) + \frac{2}{3\pi}\sum_f Q_f^2 \ln\left(\frac{M_W}{m_f}\right) + \frac{1}{18\pi}[32F-63]\ln\left(\frac{M}{M_W}\right)$$

Where $M_W \simeq 85$ GeV is the intermediate weak vector boson mass, Q_f and m_f are the charge and mass of the fermion f, and F is the number of families ($F \geqslant 3$). For this analysis to be sensible, $\alpha(M)$ is assumed to be <1. It follows that $\alpha(0)$ must be quite small. Since M is known to be $>10^{14}$ GeV, one obtains the constraint, $\alpha^{-1}(0) \geqslant 25$. In any unified scheme in which the proton decays (but not too quickly), and for which perturbative estimates are reliable, the fine structure constant cannot be large. To a greater extent than was imagined, things must be as they are.

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Direct observation of the structure of a metallic alloy glass

P. H. Gaskell & David J. Smith*

Cavendish Laboratory, University of Cambridge

C. J. D. Catto & J. R. A. Cleaver

Department of Engineering, University of Cambridge, Cambridge, UK

Under favourable circumstances, high resolution axial bright-field transmission electron micrographs can provide information on the medium-range structure of oxide glasses and amorphous semiconductors[1]. Specifically, the presence in an image of localised patterns of fringes, with the fringe spacing related by Bragg's law to the first diffraction peak, provides evidence of correlations in atomic positions over distances in the range 8–15Å. 'Amorphous lattice fringes' with these characteristics have been studied in many amorphous solids but hitherto have not been reported in glassy metals, although several attempts have been described previously[2-4]. We present here what we believe to be the first high resolution axial, bright-field micrographs with lattice resolution of an amorphous metal, taken with the Cambridge University 600 kV high resolution electron microscope (HREM)[5]. The image features obtained indicate that it is now possible to observe directly the microstructure of amorphous metals, thereby emphasising the potential of high resolution microscopy as a tool for furthering the understanding of the structure of amorphous metallic glasses.

A bright-field electron micrograph provides a representation of the atomic density of the specimen projected onto a plane normal to the electron beam. The image contrast corresponding to a Fourier component of the specimen with period Λ is the product of the scattered amplitude, represented by the square root of the structure factor and the instrumental response function. The form of the latter, commonly known as the contrast transfer function, depends on the aberration constants of the objective lens and the electron wavelength, λ. It is controlled in practice by the strength of the objective lens, as characterised by a defocus parameter[6] and modulated by envelope functions which represent the effects of non-monochromaticity of the incident electron beam and non-parallel illumination. Thus the contrast transfer function, $T(Q)$, is a damped oscillatory function of spatial frequency, $1/\Lambda$, with an amplitude which decreases rapidly for $\Lambda \leqslant 4$Å for an accelerating voltage of 100 kV, as shown in Fig. 1*a*. However, by using a very strong objective lens, it is possible to extend the CTF to better than 2Å although reversals in contrast make image interpretation difficult[7].

For crystalline specimens, the structure factor, $S(Q)$ (where $Q = 2\pi/\Lambda$) consists of a series of intense peaks sharply localised in reciprocal space. Consequently, even though the magnitude of $T(Q)$ may be very low, it is often possible to arrange that

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* Present address: High Resolution Electron Microscope, Department of Engineering, University of Cambridge, Cambridge, UK.

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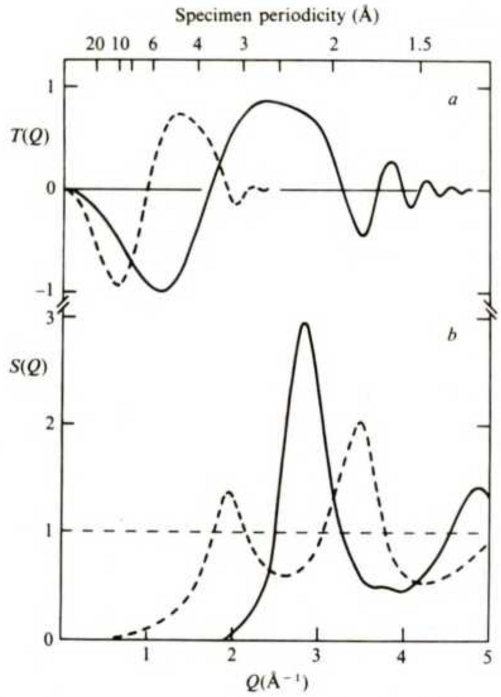

Fig. 1 *a*, Typical contrast transfer functions for electron microscopes with accelerating voltages of 100 kV (dashed line) and 500 kV (solid line). These functions are plotted for a generalised defocus value (see ref. 6) of $D = 3^{1/2}$ and with chromatic (C_c) and spherical (C_s) aberration coefficients, appropriate to typical 100 kV microscopes ($C_c = C_s = 1.8$ mm) and to the Cambridge HREM ($C_s = 3.27$ mm, $C_c = 3.33$ mm). *b*, Structure factors for *a*-Pd_4Si taken from neutron scattering data[10] (solid line) and for *a*-Ge (X ray)[11] (dashed line).

$T(Q)$ is effectively constant over the width of the peak, and that the product $T(Q) \times S(Q)^{1/2}$ is large enough for lattice fringes of adequate contrast to be observed. The intensity of the first diffraction peak for any amorphous solid is much lower than that for a crystal, and the peak is characteristically diffuse—an 'amorphous halo'. As a direct result, faithful reproduction of any periodicities in amorphous solids generally presents more difficulties and, especially at high spatial frequencies, makes extreme demands on the performance of the microscope.

To observe 'lattice' fringes in an amorphous solid, it is apparently necessary to arrange that $T(Q)$ is maximised at Q_p, corresponding to the first peak of $S(Q)$, and that a broad peak, or lobe, of the contrast transfer function, encompasses a significant fraction of the first diffraction halo[8]. For materials such as amorphous silica, carbon, germanium and silicon, $Q_p = 1.5$–2.0 Å^{-1}, these constraints can be met at 100 kV by arranging that the second or third lobe of $T(Q)$ shall coincide with Q_p. Observation of the image at 100 kV becomes almost impossible for amorphous metallic alloys with $Q_p \sim 2.8$ Å^{-1} corresponding to 2.3 Å periodicities. At such high spatial frequencies, $T(Q)$ is effectively zero, unless the spherical aberration constant of the objective lens is very low, and normally oscillates several times (giving alternate positive and negative contrast) over the width of the diffraction peak.

The shorter electron wavelength corresponding to the higher accelerating voltage (up to 0.6 MV) of the Cambridge University HREM effectively extends $T(Q)$ to higher spatial frequencies by a factor approximately proportional to $(\lambda_{500}/\lambda_{100})^{1/2} \simeq 1.6$ (ref. 9). For an accelerating voltage of 500 kV, $T(Q)$, in optimum conditions, now extends beyond $Q_p \simeq 2.8$ Å^{-1} (see Fig. 1*a*) so that the direct observation of any fringes present in amorphous metals becomes practicable. Moreover, as Fig. 1*b* shows, amorphous metals possess an important characteristic which militates in favour of clearer lattice images. Whereas the first peak of $S(Q)$ is almost invariably weak in amorphous semiconductors and oxide glasses, the first peak in amorphous Pd_4Si is strong. Also, the absolute value of the scattered intensity—proportional to the square of a weighted sum of the atomic scattering factors—is typically more than five times larger than that for amorphous carbon. Consequently, the ratio of signal from an amorphous metal foil to noise from a carbon support film, or from a layer of contamination, may be acceptably high even for very thin specimens. This is of considerable importance since any ordered domains in amorphous solids would be expected to be smaller than 15–20 Å (on the evidence of both diffraction data and dark-field microscopy). Clearly, contrast is maximised for foils of thickness near 15 Å and decreases rapidly with foil thickness thereafter, due to overlap of domains which may not be Bragg-orientated and which thereby add to the background noise.

Thin foils of a palladium–silicon alloy were prepared by flash evaporation of a crystalline alloy from a tungsten spiral onto freshly cleaved rock salt at 20 °C at a pressure of around 2×10^{-5} torr. (Other foils have been prepared by evaporation onto liquid N_2-cooled substrates at a lower pressure and these give qualitatively similar image features.) Several films of different composition were prepared and specimens selected which were amorphous, as judged by electron diffraction and confirmed by dark-field microscopy which revealed no large speckle. The composition of the foil is not known with certainty—there seems to be some loss of silicon during the flash evaporation process, but since the specimen chosen lies at the high-silicon end of the range of amorphous specimens, it was judged to contain 20–25 at. % Si. Examination at 100 kV showed that the films had a droplet morphology with particles appearing to be supported on a very thin amorphous film (silicon

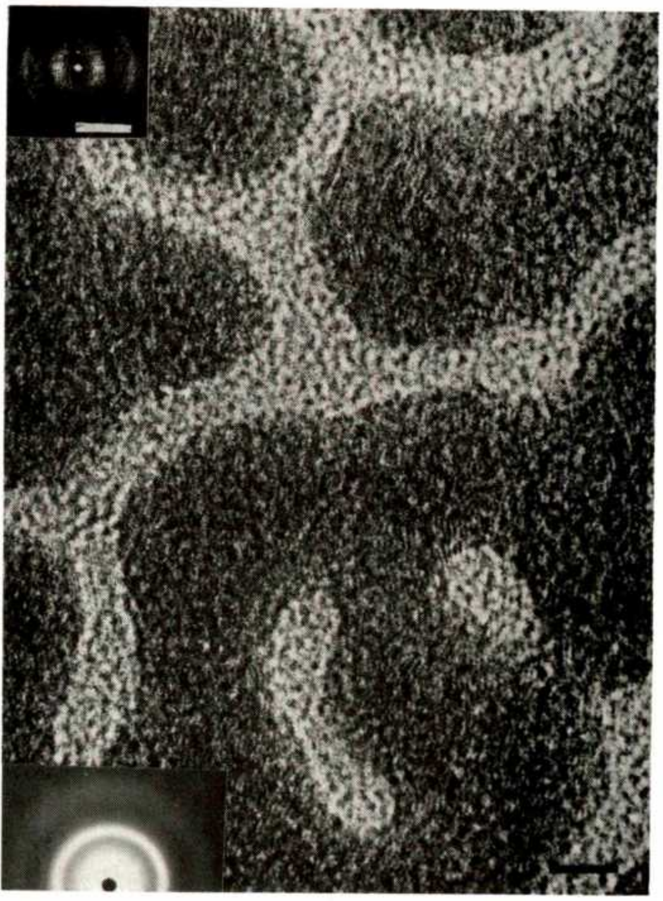

Fig. 2 High resolution image of an amorphous palladium–silicon alloy, recorded with an accelerating voltage of 500 kV. Fringe structure with a periodicity of about 2.3 Å is seen, especially in thin regions near the edge of the particles. Scale bar, 25 Å. Insets show the image power spectrum (marker represents (2.3 Å)$^{-1}$) obtained by computer processing (upper left) and the electron diffraction pattern (lower left).

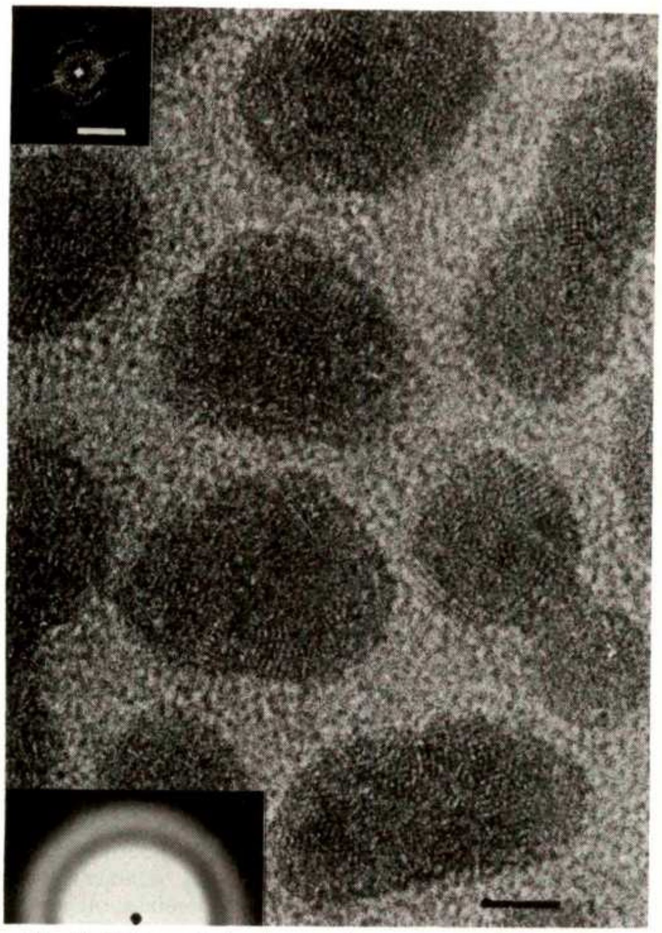

Fig. 3 High resolution image of amorphous palladium–silicon recorded in similar conditions to Fig. 2. The particles are smaller than those shown in Fig. 2 so fringe detail is more prominent.

dioxide). Some 4 Å structure was observed in this film but no high frequency detail was visible in the particles.

High resolution images recorded at an accelerating voltage of 500 kV show fringe-like features with a spacing of about 2.3 Å in the metal particles. In thicker specimens (Fig. 2) detail is most obvious, as expected, near the edge of the particles; at the centres, the structure gives the impression of randomness. Contrasting fringes are rarely seen, and then only over relatively small areas. The thickness profiles have not yet been determined, but assuming a circular cross-section, particles would be from 40 to 80 Å thick at their centres. It seems likely that the disappearance of fringe structure in the centre of particles is directly related to the overlap of Bragg-orientated regions with other, incorrectly-orientated domains.

Fringes are much more prominent in thin specimens, as evidenced by the image shown in Fig. 3. This specimen was evaporated at the same time as the thicker sample, with the thickness being varied by increasing the source–substrate distance. Although it is possible that this foil may have an essentially different structure from the thicker specimen it seems unlikely and the more pronounced structure is probably an effect of thickness. Only rarely do parallel fringe systems extend more than 20 Å in a given direction and the general impression gained from this micrograph is that each particle contains several fringe systems, some of which appear to be related by twin planes. In this sense, the particles seem to be similar to multiply-twinned particles in which lattice fringes are, however, more prominent, as identifiable multiply-twinned particles are often large and symmetrical (pentagonal or icosahedral) structures.

The fringe structures observed in Figs 2 and 3 show similarities to those seen in oxide glasses and amorphous semiconductors, in that parallel fringes are confined to local regions and the contrast is lower than that observed in single crystals of similar size. There are, however, significant differences. Fringes are not only parallel but tend to be straight, in contrast to the curved fringes often seen in oxide glasses. Moreover, the area over which fringes are seen often exceeds 100 Å^2. The electron diffraction patterns (insets in Figs 2 and 3) show the classical amorphous structure which does not appear to change with irradiation in the electron beam. Furthermore, diffraction patterns were recorded without any change of the illumination conditions from those used in first obtaining the micrographs (that is with a relatively weakly convergent beam), thus removing the possibility that the diffracted intensity from any electron-damaged region was swamped by the intensity scattered by the surrounding, unirradiated area. These figures display, perhaps more clearly than similar high resolution micrographs of amorphous semiconductors, that a locally ordered structure is not necessarily inconsistent with the impression of randomness beyond, say, 10 Å suggested by the diffraction information. The latter is an average over a specimen volume many orders of magnitude larger than that of the locally ordered domains observed in this film. A minimum conclusion appears to be that this specimen of amorphous Pd–Si possesses at least as much order as the oxide glasses and amorphous semiconductors. However, these micrographs are also inconsistent with any simple microcrystallite model.

Do the fringes necessarily imply localised order in the metallic glass? The possibility that statistically random fluctuations in a structureless medium could be imaged as fringes has been examined by several workers. Furthermore, periodic oscillations of the projected atomic density have been observed for certain orientations of (nominally) random models of amorphous metals[12]. It is not possible to relate these 'pseudo-lattice' fringes to those observed experimentally except by careful computation of axial images. Calculations by Cochran[13] and Krivanek[14] suggest, however, that fringe-like regions with projected areas as large as those shown here are unlikely to arise by chance overlap of atoms in a truly random array.

Note that the results described here relate to a single example of an amorphous alloy, and that this specimen requires more adequate characterisation. Moreover, the electron diffraction technique is one of the least accurate for obtaining even semiquantitative scattering information. Examination of a more representative range of compositions is needed before the conclusion can be drawn that these specimens, with their relatively ordered medium-range structure, are typical of the whole class of amorphous metals. However, these results clearly reveal the potential of high resolution electron microscopy as a technique for examining the structure of rapidly quenched amorphous alloys. It should also be useful in studies of the puzzling low-temperature precrystallisation phenomena which have a drastic effect on the physical properties of many amorphous metals, and for following the rather complex series of crystalline transformation.

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Control of seawater composition

D. R. Turner & M. Whitfield

Marine Biological Association of the UK,
The Laboratory, Citadel Hill, Plymouth, UK

We have previously discussed[1] the correlations between the seawater/rock partition function K_Y(sw) (mean concentration of Y in seawater/mean concentration of Y in crustal rock) and the electronegativity function $Q_{YO}[=(x_Y-x_O)^2$ where x is the electronegativity of the subscripted element][2]. The parameter Q_{YO}, which is a measure of the ionic interaction between the element Y and oxygen, was used to extimate the tendency of Y to become incorporated in the oxide-based mineral lattices which constitute most marine particulate matter. The nature of the correlations between K_Y(sw) and Q_{YO} (Fig. 2, ref. 1) led us to suggest that the partitioning of an element between seawater and crustal rock is controlled predominantly by its solid state chemistry with solution chemistry playing a secondary part. More recent estimates of the composition of mean world river water and mean crustal rock[3] significantly improve the correlations described earlier and provide fresh data on the concentrations of the lanthanides in these reservoirs. Here we compare the chemistry of the lanthanides with that of alkali and alkaline earth metals with a similar electronegativity (the 'lithium group' Li, Mg, Ca, Sr, Table 1) to confirm that solid state chemistry plays a major part in controlling the composition of seawater.

Although they have similar electronegativities there are some striking differences between the behaviour of the lanthanides and the lithium group in the world oceans (Table 1). In the log K_Y(sw) versus Q_{YO} plot (Fig. 2, ref. 1) the partition coefficients for the lanthanides are among the lowest observed and lie at the end of the main sequence established by 60 elements. The partition coefficients for the lithium group lie on a separate correlation well above the main sequence and they have K_Y(sw) values some five orders of magnitude higher than lanthanides with a corresponding electronegativity (Table 1). Furthermore the lithium group elements have long oceanic residence times and can be described as 'enriched elements'[6] as they are concentrated in the oceans relative to the world rivers (relative oceanic enrichment $RE > 0$, Table 1). The lanthanides, however, are 'depleted elements'[6] ($RE < 0$, Table 1) as they have short residence times in seawater and are effectively removed from solution on entering the world oceans. The scavenging of the lanthanides is very efficient as the concentrations of these elements in seawater are among the lowest observed for the stable elements. This is not primarily attributable to low crustal abundance since, for example, lanthanum, cerium and neodymium are more abundant than tungsten, cobalt and molybdenum in the Earth's crust[10] although the latter elements are respectively two, three and five orders of magnitude more abundant in the oceans[11].

The reason for the effective removal of the lanthanides from the oceans is not to be found in their solution chemistry. Although the lanthanides in seawater are complexed a little more strongly than the lithium group, particularly by carbonate ions (Table 1), a substantial proportion of each element is present as the free ion so that both groups of elements behave as typical (a)-cations[12] with low to intermediate polarising power[7] ($z^2/r < 11$, Table 1). The effective complexing of the lanthanides should favour higher concentrations of these elements in seawater than in river water. Solubility products do not suggest a suitable mechanism for the efficient removal of the lanthanides from seawater. Indeed, while the concentrations of some of the lithium group elements (such as Ca) are limited in seawater by the precipitation of insoluble salts, the lanthanides are several orders of magnitude below the concentration limits set by their insoluble salts (Table 2). For an explanation, therefore, we consider the differences in solid state chemistry between the two groups of elements. A key point here is the concept of 'capture' introduced by Goldschmidt[14] to describe the situation in which the incorporation of a subordinate element into a mineral lattice in place of the dominant rock-forming elements results in a significant stabilisation of the curounding crystal structure. Ion capture results when a site occupant is replaced by an ion with higher polarising power[7] (z^2/r) and/or one which can accommodate a higher coordination number. The capture of lanthanide ions by calcitic minerals is common[10,14] because the lanthanides have a higher polarising power than the alkaline earth cations of similar electronegativity (Table 1) and can accommodate higher coordination numbers[15]. Although Goldschmidt[14] originally introduced the concept of capture in discussing the formation of igneous rocks it is possible that the same process will be apparent in marine diagenesis with the adsorption of the ion onto the mineral surface being followed by ion capture. This process will

Table 1 Comparison of the properties of lanthanide and Group IA and IIA cations

								Seawater speciation¶					
Cation type	Cation	x*	r (Å)†	z^2/r	log K_Y (sw)‡	log $\bar{t}_Y$ (yr)§	RE‖	free	OH	F	Cl	SO_4	CO_3
Cerium earths	La	1.1	1.06	8.5	−5.69	3.31	−1.22	31	4	1	16	16	33
	Ce	1.05	1.03	8.7	−6.48	2.63	−1.90	22	4	1	11	21	41
	Eu	1.1	0.95	9.5	−5.63	3.53	−1.00	11	7	1	5	8	68
Yttrium earths	Gd	1.1	0.94	9.6	−5.52	3.47	−1.06	11	4	1	6	13	65
	Y	1.3	0.89	10.1	−5.95	—	—	16	14	4	7	4	55
	Lu	1.2	0.85	10.6	−4.90	3.83	−0.70	7	7	1	2	3	81
Group IIA	Mg	1.2	0.72	5.6	0.35	7.06	2.53	87	—	—	—	12	1#
	Ca	1.0	1.00	4.0	−0.59	5.98	1.45	89	—	—	—	10	1#
	Sr	1.0	1.16	3.5	−0.09	6.65	2.12	85	—	—	—	15	—
Group IA	Li	1.0	0.74	1.4	−0.92	5.71	1.18	99	—	—	—	1	—

* Ref. 4.

† Ref. 5.

‡ Recalculated from data given in ref. 3.

§ Mean oceanic residence time calculated from river input of dissolved material[6]. Residence times an order of magnitude lower are obtained if the particulate loads in river water and seawater are also taken into account[10].

‖ Relative oceanic enrichment, RE = log (concentration in seawater) − log (concentration in river water): $RE = \log \bar{t}_Y - 4.53$ (ref. 6).

¶ Whitfield *et al.*[7] using data interpolated from critical compilations of stability constants[8,9] for seawater at pH 8 and 35‰ salinity. Abundances quoted to nearest whole per cent.

# Bicarbonate.

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Table 2 Limitations set on the concentration of the lanthanides in seawater by the solubilities of their hydroxides, carbonates and phosphates

Element	Hydroxides		Carbonates		Phosphates		log [Ln]$_{obs}$ #
	log K_S (OH)*	log [Ln]$_{max}$ †	log K_S (CO$_3$) ‡	log [Ln]$_{max}$ §	log K_S (PO$_4$) ‖	log [Ln]$_{max}$ ¶	
La	21.7	−2.3	−26.4	−6.3	−18.4	−8.7	−11.2
Ce	21.3	−2.7	—	—	—	—	−10.7
Eu	18.9	−5.1	—	—	—	—	−13.0
Gd	17.0	−7.0	−25.2	−5.7	−18.3	−8.6	−12.4
Y	18.9	−5.1	−23.6	−4.9	—	—	−11.6
Lu	15.9	−8.1	—	—	—	—	−13.2

* Solubility product for the reaction $Ln(OH)_3(s) + 3H^+ \rightleftharpoons Ln^{3+} + 3H_2O$ at I = 0.7M calculated using equations by Baes and Mesmer[9].

† log [Ln]$_{max}$ = log K_S (OH) − 3 *p*H calculated for *p*H 8.

‡ Solubility product for the reaction $Ln_2(CO_3)_3(s) \leftrightarrows 2Ln^{3+} + 3CO_3^{2-}$ at I = 0.7M calculated from data by Smith and Martell[8] assuming $g_{CO_3^{2-}} = 0.1$ and $g_{Ln^{3+}} = 0.01$, where *g* is the free ion activity coefficient.

§ log [Ln]$_{max}$ = (log K_S (CO$_3$) − 3 log [CO$_3$])/2 calculated for log [CO$_3$] = −4.58 (ref. 7).

‖ Solubility product for the reaction $Ln\,PO_4(s) \rightleftharpoons Ln^{3+} + PO_4^{3-}$ at I = 0.7M calculated from data by Smith and Martell[8] assuming $g_{Ln^{3+}} = g_{PO_4^{3-}} = 0.01$.

¶ log [Ln]$_{max}$ = log K_S (PO$_4$) − log [PO$_4$] calculated for log [PO$_4$] = −9.7 (refs 11, 13).

# Seawater concentrations from Brewer[11], corrected for complexation of the lanthanide cations (Table 1).

be particularly effective in removing the lanthanides from solution where new minerals are produced in the oceans either by reverse weathering[16] or by biological processes. There is, in fact, abundant evidence for the enrichment of the lanthanides in a range of authigenic minerals including glauconites, phosphorites, foraminiferal tests and manganese nodules[10,17–20]. It is likely that the oceans are depleted in the lanthanide elements relative to the world's rivers because these effects only become apparent when the elements are held in a reservoir with a long retention time (several thousand years at least, Table 1). Evidence for the progressive incorporation of the lanthanides into phosphorites on ageing[21] supports this hypothesis.

The suggestion that ion capture controls the chemistry of the lanthanides in seawater is supported by the differences in behaviour noted between the lanthanides themselves. In comparison with chondritic (non-ferrous meteoric) minerals the lighter lanthanides (the 'cerium earths' La–Eu) are enriched in preference to the heavier lanthanides (the 'yttrium earths', Gd–Lu and Y) in most hydrogenous minerals[10]. This preferential enrichment probably arises because the effect of the increasing polarising power of the cation (z^2/r, Table 1) on going from La to Lu is outweighed by the higher coordination number which the larger, lighter cations are able to accommodate in oxide lattices[15], thus favouring their capture. As a result of their preferential enrichment in mineral structures the 'cerium earths' have significantly shorter residence times ($\bar{t}_Y$) in the oceans and lower partition coefficients (K_Y(sw)) than the 'yttrium earths' (Table 1). The plot of K_Y(sw) against Q_{YO} (Fig. 2, ref. 1) shows that these differences are adequately represented by the electronegativity function, Q_{YO}.

Thus, the chemistry of the lanthanides supports our original deduction[1], based on the distribution of the elements along the K_Y(sw) versus Q_{YO} plot, that the overall composition of seawater is controlled by the solid-state chemistry of the elements. The comparison between the lanthanides and the lithium group also clarifies the mechanism by which solid-state control is achieved in the case of the electropositive elements.

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Iron–nickel superstructure in metal particles of chondrites

J. Danon, R. B. Scorzelli & I. Souza Azevedo

Centro Brasileiro de Pesquisas Físicas, Av. Wenceslau Braz, 71-Rio de Janeiro, Brasil

M. Christophe-Michel-Lévy

Laboratoire de Minéralogie-Cristallographie, associé au CNRS-Université Paris VI, France

Ordered solid solutions (superstructures or superlattices), in which atoms of one kind segregate into a particular set of lattice positions, are usually obtained by slow cooling at the critical ordering temperature. However, the equiatomic Fe–Ni ordered phase (superstructure Llo) has been obtained only by radiation-induced ordering[1], since the diffusion rates of the metallic atoms are very slow at the ordering temperature T_c = 320 °C (ref. 2). The Fe–Ni ordered phase has recently been detected by Mössbauer spectroscopy and X-ray diffraction in the taenite of the iron meteories Cape York (III A), Toluca (I)[3] and reported as a major constituent of the Ni rich ataxite (35% Ni) Santa Catharina[4]. We report here Mössbauer spectra, scanning electron microscopy and X-ray measurements, which show that the metal particles of the chondrites Saint-Séverin (LL6), Lake Labyrinth (LL6) and Parambú (polymitic LL breccia) contain large proportions of this Fe–Ni superstructure and that the phase is present in different amounts in the chondrites L'Aigle (L6), Peetz (L6), Shaw (L6), Bjürbole (L4) and Allegan (H5).

The identification of the Llo superstructure in Fe–Ni alloys by Mössbauer spectroscopy is based on the fact that the ordered phase exhibits an asymmetric 6-line spectrum due to a quadrupole splitting arising from the non-cubic environment of the Fe atoms in this structure. Detection by X-ray diffraction is more difficult due to the very weak intensity of the superstructure lines[5].

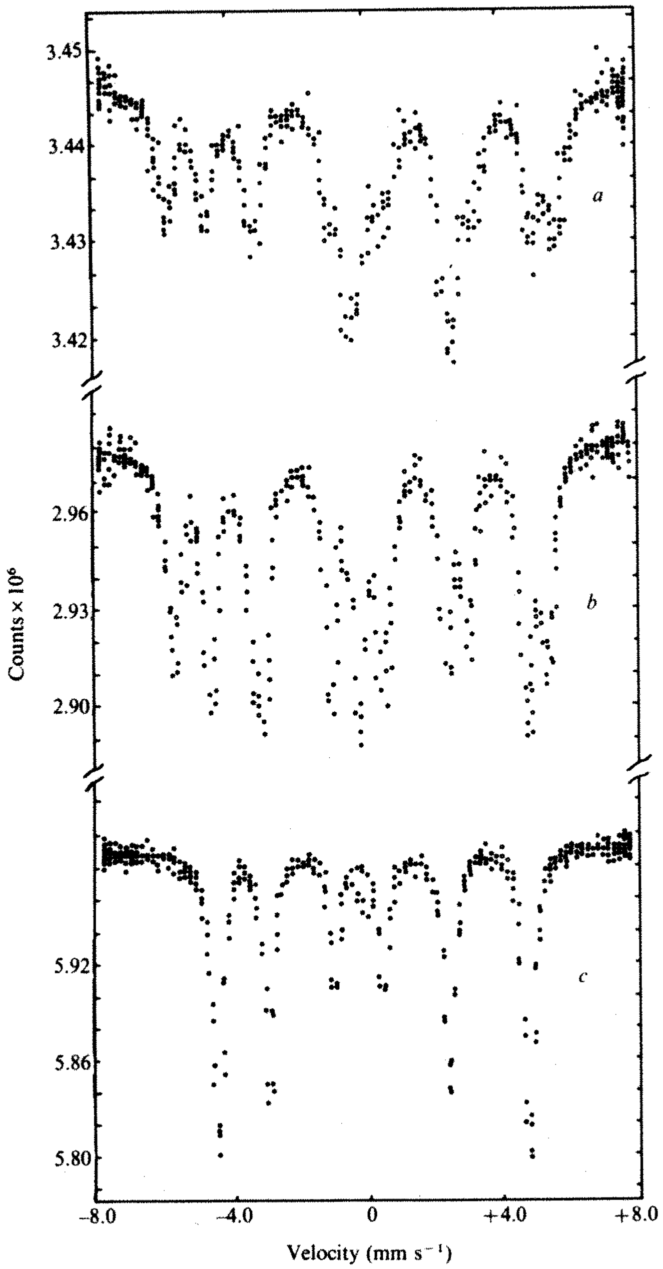

Fig. 1 Mössbauer spectra of St Séverin metal fraction at room temperature recorded with a sinusoidal drive and HP multichannel analyser (1,024 channels). The data were fitted with a 370/145 IBM computer. *a*, Untreated metal fraction magnetically separated and sieved to 40–80 μm. *b*, Metal fraction (80–150 μm) after HF treatment. *c*, Ordered phase in pure form extracted from metal fraction. The quadrupole effect is seen in the asymetric position of the outer lines. From spectrum *c* one identifies the lines corresponding to the ordered phase in the upper spectra *b* and *a*.

Chondrites exhibit complex Mössbauer spectra[6] which arise from the superposition of: (1) quadrupole interaction from the paramagnetic iron silicates which yields two pairs of doublets. (2) Magnetic hyperfine interaction from the α-phase (kamacite) or the α_2-phase (martensite), γ-phase (taenite) and troilite, which yields three six-line spectra resulting in a total of 18 partially overlapping lines. (3) A single central line arising from paramagnetic γ-phase (taenite).

With magnetically enriched samples relatively large amounts of silicates and troilite still remain attached to the metallic phase. Only in a favourable case such as St Séverin, which contains a large proportion of the ordered phase, was it possible to detect the superstructure by Mössbauer spectroscopy without further purification of the sample (Fig. 1*a*).

We found that a separation of the metals from the silicates (up to about ~95%) and the troilite can be achieved by boiling the metallic fraction in concentrated HF (40%) for a few minutes and submitting the residue to a magnetic separation. This treatment does not affect the metallic phase, as we observed in experiments with several Ni-rich Fe-Ni alloys. Moreover the Ni/Fe ratios estimated from the areas corresponding to the several metallic phases observed in the Mössbauer spectra (Fig. 1*b*) are in reasonable agreement with those reported by chemical analysis.

Table 1 lists the hyperfine parameters derived from the Mössbauer spectra of the chondrite metals in which the ordered phase has been observed, as well as the proportions of the Fe–Ni alloy phases in these meteorites.

The relatively large Ni/Fe ratio found in the metallic phase of the LL chondrites favours the formation of the Llo superstructure which also seems to be common in the L chondrites, although in smaller proportions. The presence of the ordered phase in the H chondrite Allegan was confirmed by a Debye–Scherrer diagram (filtered CoKα) of an untreated metal particle presenting a cloudy taenite border which showed the α-phase, the γ-phase and (100), (110) and (210) superstructure lines (J. Laugier, personal communication).

Within the sensitivity of the Mössbauer technique (up to a few per cent) no evidence for the ordered phase was found in the

Table 1 Hyperfine parameters of the chondrite metal fraction

Chondrites	α-phase				Ordered γ-phase					γ-phase (paramagnetic)		
Metal fraction	*IS* (mm s^{-1})	*H* (kOe)	*W* (mm s^{-1})	*A* (%)	*IS* (mm s^{-1})	ΔE_Q (mm s^{-1})	*H* (kOe)	*W* (mm s^{-1})	*A* (%)	*IS* (mm s^{-1})	*W* (mm s^{-1})	*A* (%)
St Séverin	−0.09	343	0.43	39.9	−0.07±0.05	+0.20±0.01	289	0.43	50.8	−0.16	0.38	9.5
Lake Labyrinth	−0.11	322	0.76	50.7	−0.03±0.03	+0.13±0.05	288	0.51	36.6	−0.16	0.45	9.8
Parambú	−0.07	340	0.50	37.8	−0.08±0.02	+0.16±0.02	294	0.50	42.7	−0.12	0.46	9.8
L'Aigle	−0.10	338	0.45	74.1	−0.14±0.03	+0.16±0.05	302	0.45	16.0	−0.13	0.46	9.8
Bjürbole	−0.09	339	0.44	85.2	−0.04±0.03	+0.24±0.03	298	0.44	10.9	−0.14	0.33	3.9
Allegan	−0.09	335	0.38	64.5	−0.02±0.04	+0.22±0.03	287	0.38	17.5	−0.06	0.43	10.7
Peetz	−0.10	343	0.57	86.3	−0.19±0.07	~0	300	0.57	7.8	−0.10	0.78	5.9
Shaw	−0.10	341	0.48	62.2	−0.09±0.03	~0	298	0.48	17.4	−0.03	0.67	9.5
Enriched ordered phase												
Parambú	—	—	—	—	−0.07±0.007	+0.12±0.01	288	0.49	70.7	—	—	—
St Séverin	—	—	—	—	−0.07±0.002	+0.21±0.01	287	0.35	100.0	—	—	—

IS, The isomer shift relative to a 25 mC Co^{57} in Rh (error ± 0.01 in α-phase and ±0.04 mm s^{-1} in paramagnetic γ-phase); ΔE_Q, quadrupole splitting; *H*, magnetic field (±5 kOe); *W*, linewidth (±0.03 mm s^{-1} in the α and ordered phase, and ±0.3 mm s^{-1} in the paramagnetic γ phase); *A*, area (±5%), <10% iron silicates are present in Shaw, Allegan, Parambú, and <5% in the others. The ordered phase in Parambú was enriched up to 70% and up to ~100% in St Séverin.

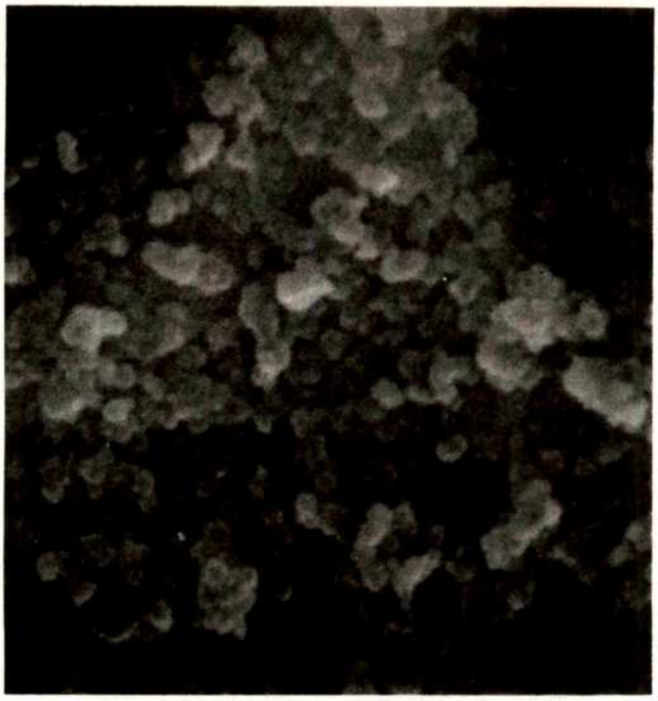

Fig. 2 Scanning electron micrograph (×20,000) of the Fe–Ni ordered phase from St Séverin.

metal phases of the H chondrites Nadiabondi, Menow, Ipiranga, Marilia, São José do Rio Preto, Avanhandava and Estacado. The X ray of a Nadiabondi metal grain, however, exhibited a faint (100) superstructure line.

We were able to isolate the Fe–Ni ordered alloy as a single phase constituent. In a Cu^{2+} solution both Fe(0) and Ni(0) metals are dissolved[7] but the rate of dissolution is slower for the ordered phase. A fraction in which this phase is enriched can be obtained by treating the metallic fraction (after HF) for several minutes in a $CuCl_2/2KCl$ solution. Cu(0) is next eliminated from the metal residue with concentrated ammonia. A sample extracted from St Séverin exhibits a Mössbauer spectrum which corresponds to an essentially pure ordered phase (Fig. 1*c*). The composition of the alloy in this sample was found to be Fe(60%) and Ni(40%), in the domain of stability of the Llo superstructure[2].

Scanning electron microscopy showed an assembly of small structures (Fig. 2), similar to those reported by Scott in crystallographic studies of cloudy taenite[8], with sizes between 1,500 and 3,000 Å and quite homogeneous metal composition.

The parameter $a_0 = 3.579 \pm 0.001$ Å obtained by X-ray diffraction of this sample is that reported for the ordered phase[9,10] and is smaller than that corresponding to the disordered alloy $a_0 = 3.596 \pm 0.001$ Å. With a CoKα radiation in a 24-h exposure the (100), (110), (210), (211) and (221) weak superstructure lines were detected in the Debye–Scherrer diagram[11].

The value of the quadrupole splitting of the ordered phase extracted from Parambú ($+0.12 \pm 0.01$ mm s^{-1}) is significantly smaller than that observed with St Séverin ($+0.21 \pm 0.01$ mm s^{-1}). Parambú is a polymitic breccia with evidence of a shock event[12] whereas St Séverin presents little evidence of shock. This suggests that the shock event, either from pressure effects[13] or by impact heating, tends to disorder the Fe–Ni superstructure ($T_c = 320$ °C), as indicated by the decrease in the value of the quadrupole splitting[14]. This would explain the low value ($\Delta E_Q = +0.13 \pm 0.05$ mm s^{-1}) observed with Lake Labyrinth, which according to Taylor and Heymann[15] is a heavily shocked chondrite (class C(H)).

The cases of Peetz and Shaw are particularly interesting since their Mössbauer spectra corresponds to the α (or α_2) phase, as in the other chondrites, and a ferromagnetic γ phase with same hyperfine parameters of the superstructure but largely disordered ($\Delta E_Q \sim 0$). The metallic phases in Peetz indicated evidence of shock, but only moderate reheating[15]. The relatively large negative value of the ^{57}Fe isomer shift observed with Peetz is compatible with a pressure effect above 200 kbar[16].

Recent studies indicate that Shaw is a complex meteorite which experienced an unusual event, superimposed on a normal slow cooling history, probably connected with an intense shock[17–20]. The destruction of the superstructure in Shaw could arise by impact reheating of the meteorite to temperatures above 320 °C and rapid cooling to lower temperatures which prevented the subsequent ordering of the Fe–Ni alloy.

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Ionospheric–magnetospheric contributions to storm-time magnetic field changes near dip equator

C. A. Reddy, V. V. Somayajulu & K. S. Viswanathan

Space Physics Division, Vikram Sarabhai Space Centre, Trivandrum 695 022, India

Experimental identification of the electric current systems in the ionosphere and magnetosphere during magnetic storms is a fundamental problem. We report here a new approach to resolving the relative contributions of ionospheric and magnetospheric currents to the storm-time geomagnetic field variations at equatorial latitudes. In this approach, the VHF backscatter radar-measured electric field changes in the equatorial electrojet and the simultaneous surface magnetic field changes are analysed to determine quantitatively the ionospheric contribution ΔF_I to the storm-time geomagnetic field perturbation ΔF_D. The magnetospheric contribution ΔF_M is then determined from:

$$\Delta F_D = \Delta F_I + \Delta F_M \qquad (1)$$

This method has yielded extremely interesting results for the magnetic storm of 15 February 1978. It has a great potential for providing valuable new insights into the nature and time evolution of the complex storm-time current systems in the ionosphere and magnetosphere.

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A coherent VHF backscatter radar and a proton precession magnetometer (PPM) were used for storm-time observations at Thumba near Trivandrum (dip 56′ S; lat, 8.5° N; long, 77° E). The two experimental systems are described elsewhere[1,2]. Later improvements have enabled us to record the radar digital data on a magnetic tape and process it on an IBM 360/44 computer. The data recorded at 1-min intervals are used here. As the dip angle is very small (56′ S) the approximation $\Delta H \simeq \Delta F$ is used, where ΔH is the perturbation in the horizontal component of the magnetic field. All interpretations given here are based on this approximation. For the radar geometry at Thumba, the mean Doppler frequency $\bar{f}_D$ is negative when the drift of the ionisation irregularities (and electrons) is westward under the action of an eastward electric field. From the observed $\bar{f}_D$ fluctuations, the fluctuations of the electron drift velocity (which are directly proportional to electric field fluctuations) can be determined from standard theoretical relationships[3].

Figure 1 shows the observed variations with time of the geomagnetic field perturbation ΔF, the backscatter signal strength V and the mean Doppler frequency $\bar{f}_D$ of the radar signals on 15 February 1978. A sudden commencement storm started at 03.17 IST (82.5° EMT). The most remarkable features in Fig. 1 are: (1) a delay of about 1.5 h in the first appearance of the radar signal (at 0905 h) indicates that the normal eastward electric field is suppressed between 0730 and 0900 h; (2) the large in-step fluctuations of ΔF, $\bar{f}_D$ and V between 0905 and 1430 h of the storm initial phase; (3) a large main phase decrease of ΔF to negative values between 1530 and 1630 h, even while $\bar{f}_D$ is increasing shows that a magnetospheric current is responsible for the large negative value of ΔF; (4) a repeated disappearance of the radar signal for durations of 20–30 min, indicating the reversals of normal daytime eastward electric field in the electrojet; (5) the disappearance of large amplitude fluctuations at about 1800 h after the storm recovery phase has set in. Other interesting details of the observations shown in Fig. 1 will be discussed later. The time variations of ΔF, $\bar{f}_D$ and V on 16 February 1978 were also recorded during the recovery phase of the storm.

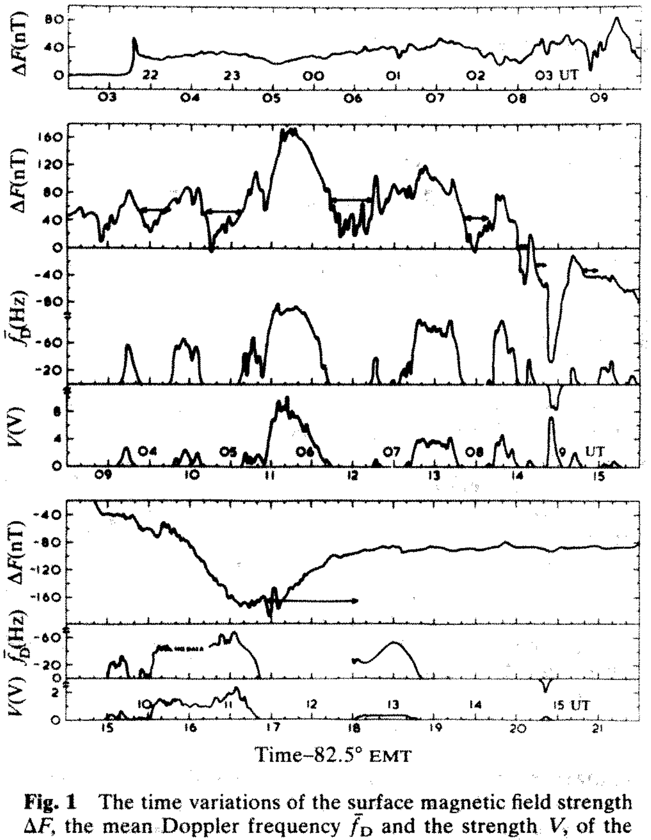

Fig. 1 The time variations of the surface magnetic field strength ΔF, the mean Doppler frequency $\bar{f}_D$ and the strength V, of the VHF backscatter radar signals on 15 February 1978. The horizontal arrows (↔) mark the periods during which the radar signals disappeared. $A_p = 48$. Time is 82.5° EMT.

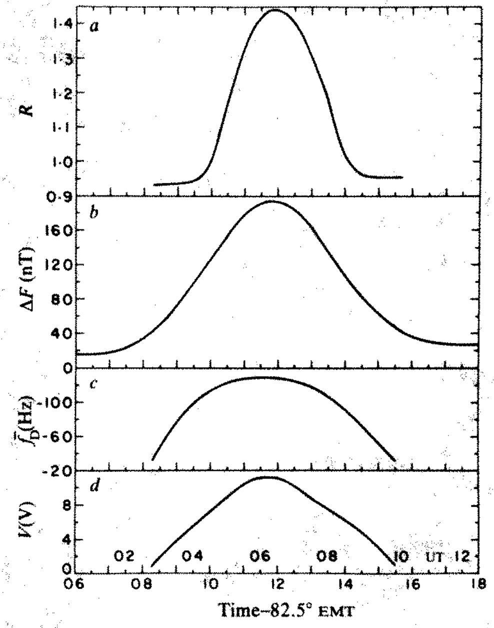

Fig. 2 The time variations of ΔF, $\bar{f}_D$ and V on 11 February 1978 (a quiet day with $A_p = 5$). $R = \Delta F/\bar{f}_D$ is also shown (a).

The interesting and informative experimental observations shown in Fig. 1, along with similar observations on a quiet day, have been used to quantify the ionospheric and magnetospheric contributions to the storm-time surface magnetic field perturbations at Thumba. Figure 2 shows the time variations of ΔF, $\bar{f}_D$ and V on 11 February 1978 which is a quiet day with $A_p = 5$. In Fig. 2a, the ratio $\Delta F/\bar{f}_D$ is shown as R,

$$R = \Delta F/\bar{f}_D \quad (\mathrm{nT})\,(\mathrm{Hz})^{-1} \tag{2}$$

Using the suffixes Q and D respectively for quiet and disturbed day values, the approximation $\Delta F_Q = \Delta F_I$ has been used instead of $(\Delta F_I)_Q = (\Delta F - D_{st})_Q$. A later check on 11 February D_{st} values shows that R value may be in error by 10–20% due to the non-subtraction of D_{st} values. Note that

$$\Delta F_I = k_1 \Sigma_3 E_y \quad \text{and} \quad \bar{f}_D = k_2 \bar{\mu} E_y \tag{3}$$

Here, $\bar{\mu}$ is a mobility parameter involving the collision and gyro frequencies of ions and electrons; k_1 and k_2 are constants of proportionality which are independent of the medium; E_y is the east–west electric field which varies very little with height if it is a global scale field and is assumed to be height-independent; Σ_3 is the height-integrated Cowling conductivity; and the bar denotes a weighted average over that height region which contributes to the backscatter signal. From equations (2) and (3), we can write

$$R = (k_1/k_2)\Sigma_3/\bar{\mu} \tag{4}$$

Thus, the variation of R should represent the variation of Σ_3, since k_1 and k_2 are constants and $\bar{\mu}$ is likely to have negligible variation with time of the day compared with that of Σ_3.

If it is assumed that at any given local time the change in Σ_3 from a quiet day to a disturbed day is much smaller than that in the electrojet electric field, then ΔF_I on a disturbed day can be deduced from

$$\Delta F_I = R_Q(\bar{f}_D)_D \tag{5}$$

Then the magnetospheric contribution ΔF_M is determined from equation (1). Obviously, the errors in ΔF_I and ΔF_M deduced in this way are proportional to the difference $(R_D - R_Q)$. The fractional change in R from quiet to disturbed day is not likely to exceed 15–20% most of the time while the ΔF_I and ΔF_M changes from quiet to storm day are in the range of 50–200%. Pending a further quantitative study of the $(R_D - R_Q)$ values, we assume that R_Q values can be used on a disturbed day to determine storm time ΔF_I and ΔF_M. Ideally, we need at least 4 or 5 quiet days' observations to arrive at the quiet day mean value of R for a given month. The induced Earth current effects are not separated out in the present analysis.

The ionospheric and magnetospheric contributions to the surface magnetic field perturbations during the 15 February storm have been estimated using the method outlined above. The results are shown in Fig. 3, where the chain curve represents the quiet day variation of ΔF and the dotted portions of the ΔF_M curve correspond to the times of disappearance of backscatter signals. If the eastward current and hence ΔF_I is assumed to be zero at such times, then $\Delta F_M = \Delta F_D$ at such times and this is the lower limit to ΔF_M. On the other hand, the broken portions of the ΔF_I and ΔF_M curves correspond to those times at which backscatter signals on the disturbed day are available but not on the quiet day, and hence an extrapolated value of R has been used. R is assumed to have its asymptotic value of 0.96 at such times.

The ΔF_I behaviour between 0900 and 1500 h indicates that the electrojet electric field and current are fluctuating violently during this time, with the average levels being rather low compared with quiet day values. The time variation of ΔF_I between 1500 and 1900 h shows that there is an enhancement of the eastward electric field and current values over the quiet day (11 February) levels in this time sector.

Consider the behaviour of ΔF_M. The circles in Fig. 3 represent the smoothed values of ΔF_M obtained through a 60-min running average of 1-min values. Between 0900 and 1400 h, ΔF_M is essentially positive and is subject to strong oscillations of 4–6 min period and to longer period (20–40 min) perturbations like ΔF_I. The 4–6 min oscillations are apparently hydromagnetic waves originating in the magnetosphere and propagating to the equatorial ionosphere[4]. In fact, detailed examination has shown that the short period oscillations as well as the longer period bay type perturbations in ΔF_I and ΔF_M are in antiphase with each other. Such simultaneous phase-coherent perturbations in ΔF_I and ΔF_M indicate that the magnetosphere, auroral ionosphere and equatorial ionosphere together respond as a single electrodynamic system to storm-time disturbance forces; and the interactions and feedback effects within this system cannot be ignored in any study of the physical processes. This and other features, when studied in detail, may provide important clues to the nature of magnetosphere–ionosphere responses and interactions during storms and substorms.

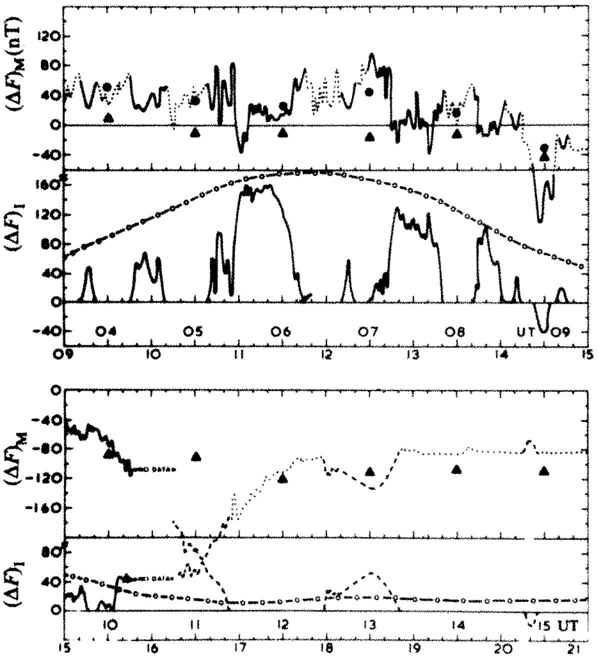

Fig. 3 The time variations of ΔF_I and ΔF_M on 15 February 1978. Solid line, ΔF_D; O—O, ΔF_Q; ▲, D_{st}. $A_p = 48$.

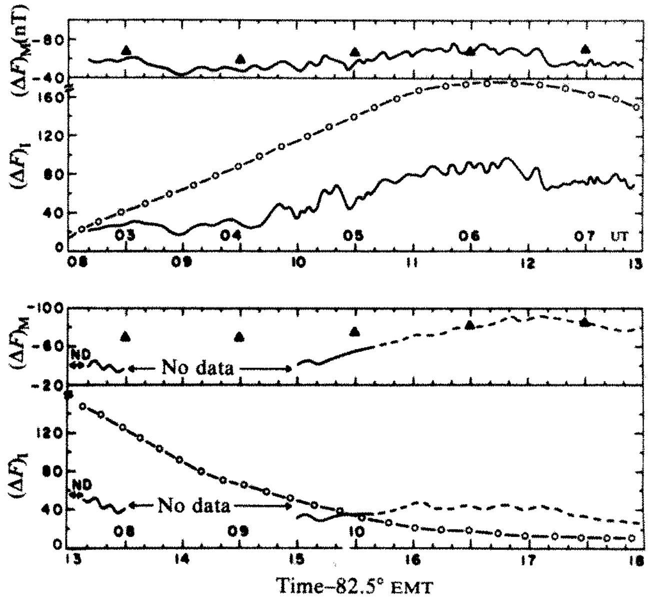

Fig. 4 The time variations of ΔF_I and ΔF_M on 16 February 1978. Solid line, ΔF_D; O—O, ΔF_Q; ▲, D_{st}. $A_p = 11$.

Moreover, the agreement between the D_{st} values and ΔF_M values is not good between 0900 and 1430 h. Apparently, the D_{st} analysis cannot correctly bring out the magnetospheric contribution to ΔF during this initial phase which is dominated by short-period oscillations and irregular perturbations.

The magnetospheric contribution ΔF_M begins to attain large negative values rather rapidly from 1520 h onwards. Significantly, the short-period oscillations and bay-type disturbances come to an abrupt stop during this phase of the storm. However, the large rapid decrease of ΔF_M and its recovery between 1600 and 1730 h are not shown up by the D_{st} analysis. But, the D_{st} values and ΔF_M values show a remarkable agreement after 1730 h. Obviously, during the late afternoon–evening sector, the surface magnetic field perturbation is caused predominantly by magnetospheric currents; and ΔF_M continues to be large throughout the night of 15 February.

Similar analysis has been done for 16 February, and the main results (shown in Fig. 4) for the recovery phase are: (1) During the recovery phase of the storm ΔF_I is still well below the quiet day value until 1400–1500 h IST; but ΔF_I clearly exceeds (by a factor of 2 or 3) the quiet day value between 1600 and 1800 h. These features indicate that a disturbance associated westward electric field persists in the equatorial electrojet during the recovery phase in the morning–noontime sector, followed by an enhanced eastward electric field in the late afternoon–evening sector. (2) On 16 February there is an excellent agreement in general between D_{st} and ΔF_M values (which are high), implying that once the symmetric ring current is well developed as a dominant feature of the magnetosphere, the procedure used for deducing the D_{st} values is able to reproduce its behaviour quite well.

The present method opens up new possibilities in quantitative studies on the equatorial electrojet current changes during different phases of magnetic storms. However, the quantitative accuracy of the present method can be improved only through further refinements in the methods of radar measurement and the data analysis. For example, the variation of R in Fig. 2 has a gross resemblance to the expected variation of Σ_3 but it shows important deviations as well. The causes of the deviations are being investigated. Similarly, we are aware[5] that local winds with vertical shear can modify and distort the height structure of the electrojet, but the wind-generated electric fields are usually much weaker than the large storm-time electric field changes. On the other hand, the excellent agreement between ΔF_M and D_{st} values over long periods of storm-time (particularly after the development of the ring current) cannot be fortuitous, and this lends support to the assumption that change in the R value from quiet to storm day is small compared to the changes in E_y. In any case the estimated errors in ΔF_I and ΔF_M can be as large as 25% in the first attempt, although we believe that the errors are much less for a considerable period of the storm-time.

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Do sponges help hold coral reefs together?

Janie L. Wulff & Leo W. Buss

Evolutionary Biology Group, Department of Biology, OML Yale University, New Haven, Connecticut 06520

The growth and form of coral reefs is the result of a complex balance between rates of carbonate accretion and carbonate loss. Reef organisms have traditionally been classified, with respect to their role in this balance, as primary frame-builders, frame-cementers, biological eroders or sediment producers[1,2]. Scleractinian corals are the primary frame-building organisms in most modern reef environments. The growth of many such corals generates large volumes of unoccupied cryptic space. Successful reef construction occurs if this space is infilled with sediment, this sediment is cemented by frame-cementing organisms, and the resulting complex is subsequently lithified[3–5]. As this lithification process may be slow, frame material is highly susceptible to becoming separated from the reef framework due to the action of physical disturbance (such as wave shock) before permanent consolidation[6–10]. We present here experimental evidence that demosponges (which may be second only to scleractinian corals in space occupancy on many Caribbean reefs) play an important role as interim binders of unconsolidated frame material, a process which is expected to increase rates of carbonate accretion.

Although sponges may bind carbonate to the reef frame in several reef zones (for example, interstices of ramose corals and carbonate rubble), our investigations have focused on Caribbean deep fore-reef environments. Deep fore-reef zones are characterised by steeply sloping sand plains dotted with small patch reefs or larger reef pinnacles and are bounded to the seaward by vertical rock walls which drop to considerable depths. Scleractinian corals growing in this zone generally exhibit a foliaceous growth morphology. Such forms are especially susceptible to disengagement from the reef since their attachment zones are small; their undersurfaces are largely devoid of live coral tissue, leaving them open to recruitment of boring organisms; and they extend off the reef rather than resting on it, making them unstable once their attachment zone is weakened[11]. Foliaceous corals have become separated from the reef frame by the forces exerted by a feeding parrotfish, by the actions of storm-induced wave shock[6–10], by the fins of a careless scuba diver, or simply due to collapse under their own weight[11]. Once separated from the reef, corals cannot reattach and are rarely consolidated.

Non-burrowing sponges characteristic of deep fore-reef environments are of two general types: those which inhabit the holes and crevices in the reef frame and those which inhabit exposed reef substrata. Cryptic sponges (such as *Agelas clathrodes* (Schmidt)) commonly have zones of attachment which include both the undersurface of foliaceous corals and the reef frame itself. The porosity of the reef frame may cause many of these sponges to extend from one attachment spot on the undersurface of a coral, through the reef frame to an attachment zone on another coral.

Sponges, including those which inhabit open reef substrata, have been observed to attach to one another without harm to either side[12,13]. Experiments performed on the barrier reef of Belize show that several such sponges may also kill small patches of coral tissue on contact and bind to the exposed coral skeleton. Growing tips of at least five individuals of each of 12 sponge species were severed with a diving knife and attached to living agariciid corals with cotton twine. As controls, pieces of commercial bath sponge were attached to living corals. The contacts were examined after one week for evidence of coral tissue death and sponge–coral binding. Of the 12 species, 10 killed a small patch of coral tissue and bound to the skeleton (Table 1). The two remaining species killed small patches of coral. The bath sponge had no observable effect. The morphological flexibility of sponges, their ability to attach to other living sponges and corals, and their ability to attach to clean substrata with any part of their colony results in the formation of anastomosing networks of several sponge species which have many attachment points on different coral colonies and the reef frame.

Corals, although far more constrained in morphological flexibility by their rigid skeletons, can also modify their forms in the presence of certain sponges. We have observed colonies of *Agaricia agaricities* (Linn.), *Agaricia lamarcki* (E & H), and *Helioserius cucullata* (E & S) which have encircled the tissue and even attachment zones of nearby sponges with skeletal material. These observations may simply reflect a passive response of the coral to any obstruction within the path of growth.

Table 1 Sponge–coral binding experiment

Sponge species	Total no. of individuals tested	Total no. killing tissue	Total no. binding to coral
Agelas clathrodes (Schmidt)	5	5	0
Agelas conifera (Schmidt)	5	5	5
Agelas sceptrum (Lam.)	5	5	5
Aplysina cauliformis (Carter)	5	5	5
Callyspongia sp.	7	7	7
Ectyoplasia ferox (D & M)	10	8	8
Haliclona rubens (Palles)	30	25	15
Iotrochota birotulata (Higgin)	5	5	5
Niphates erecta D & M	10	5	0
Smenospongia aurea (Hyatt)	5	3	3
Thalysias juniperina (Lam.)	5	2	2
Unidentified	7	7	5
Control	5	0	0

Experiments were performed while operating from the RV Alpha Helix. Two locations, one each in the lee of Glovers and Lighthouse Atolls at depths of 20–30 m, were studied. The Lighthouse Atoll site is 1 mile south of Long Cay and the Glovers site on the reef pinnacle, Movie Mountain, located on the southwestern extreme of the atoll. The experiment does assist with the understanding of the mechanism (for example, allelopathy and abrasion) by which sponges kill coral tissue. As these results represent the number of individuals binding to corals in only 1 week, they probably underestimate the actual capacity of these sponges to bind to corals.

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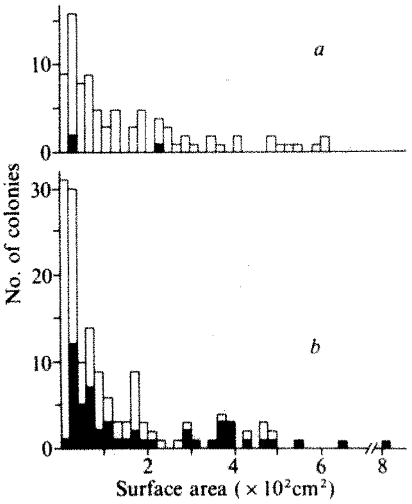

Fig. 1 Size against frequency distribution of foliaceous corals originally extant on *a*, unmanipulated reefs and on *b*, manipulated reefs. Shaded histograms represent those corals which became separated from the reefs. Patch reefs selected were comparable in size and sponge–coral faunal development. Specific data on patch morphology, sponge–coral distributions, and sponge–coral identifications are available from the authors. Coral surface area measurements were made by approximation to regular polyhedrons. Corals which immediately fell off the manipulated reefs when sponges were removed constituted <2% of the total corals manipulated (and are not included in the analysis) indicating that any disturbance to the reefs involved in removal of sponges was minimal. Data presented are clumped for all patches over the two observation periods. There was negligible sponge regrowth on manipulated reefs over the experimental period.

The hypothesis that sponges bind corals to the reef frame has previously been considered[14–17]: for example, Goreau and Hartman stated that "large encrusting sponges often support and hold such corals in place long after their original holdfasts have been eroded away". To test this hypothesis we removed sponges from patch reefs on the fore-reef slope of Marsagantupo Island, San Blas, Panama, and monitored how the coral survived. The experiments were performed on the leeward side of the island at depths ranging from 13 to 20 m. Marsagantupo Island is exposed directly to the Caribbean Sea. The first 3 months of the experimental period correspond to the dry season in Panama, the period of greatest swell. Although physical data are scant for this region, the Caribbean coast of Panama is regularly exposed to seas up to 10 ft (ref. 18). Eight small patch reefs were selected and all corals mapped as to their position on the patch. All sponges, with the exception of borers and surface encrusters of <1 cm vertical relief, were carefully removed by hand or with a diving knife from half of the reefs. The patches were revisited at three and six months after the removal and all corals remapped.

Figure 1*a* shows the size (surface area live tissue) frequency distribution of foliaceous corals originally extant on unmanipulated reefs and Fig. 1*b* shows that for manipulated reefs. Control reefs lost 2% of their colonies (2% surface area) within three months and 4% of their colonies (3% surface area) after six months; whereas manipulated reefs lost 26% of their colonies (29% surface area) within the first three months and a total of 40% of their colonies (46% surface area) after six months. The high mortality of small colonies, many of which had no direct contact with any sponge, reflects the need of juvenile corals for stable substrata on which to grow. Those small corals which have been asexually reproduced (or which have settled) on top of larger colonies are clearly as dependent on sponge binding as their older counterparts.

The traditional interpretation of the role of sponges in the reef carbonate balance has emphasised the destructive influence of a small group of biological eroders. Although the influence of biological erosion or the binding capacities of other reef sponges should not be underestimated. Demosponges clearly should be taken into account in the reef carbonate balance equation.

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Bryozoan overgrowth interactions—the interdependence of competition for space and food

Leo W. Buss

Evolutionary Biology Group, Department of Biology, OML,
Yale University, New Haven, Connecticut 06520

Space has been demonstrated repeatedly to be a limiting resource to sessile suspension-feeding invertebrates[1–7,15], whereas the question of whether food resources are ever limiting has long been a controversial topic[8,9]. On the basis of previous research and in the virtual absence of any mechanistic understanding, it is commonly assumed that interactions can be interpreted as competition for spatial resources alone. This assumption is clearly unacceptable. Whereas a mobile organism might feed in one location rich in prey, spawn in another safe from predators of its young, and hide in yet another inaccessible to its own predator, a sessile organism cannot separate, spatially or temporally, its demands for food to eat and space on which to live. For the sessile organism, access to food implies access to space and vice versa. Consequently, the empirical observation of competition for space does not justify the assumption that competition for food is unimportant. I report here that competition for food among suspension feeders can occur and may, in fact, provide a mechanism by which sessile organisms compete for space.

Competition between colonial, encrusting organisms occurs whenever the space available on a particular hard substratum is reduced sufficiently for the lateral margins of colonies to come into contact[10]. These organisms grow[11,12] and interact[1,13–19] along their colony margins exclusively and competition is commonly characterised by overgrowth (Fig. 1*a*). The physical overgrowth of one organism by another is commonly irreversible, resulting in the death of the overgrown tissues[1,13–19]. In that the overgrowing organism now occupies the space which the overgrown organism had occupied previously, such interactions constitute competition for space[20]. Although little is known of the mechanisms of overgrowth, they must involve differential growth along interspecific colony margins. The organism that grows fastest here will overgrow its competitor, perhaps by

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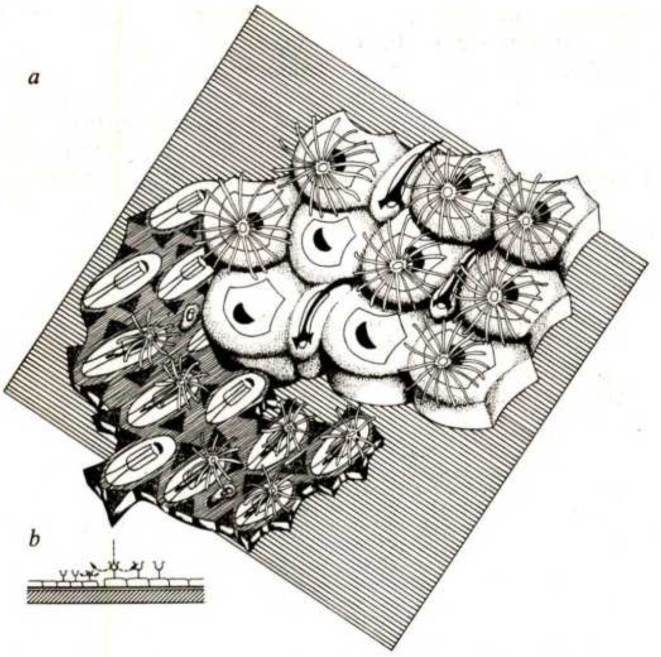

Fig. 1 The overgrowth phenomenon. *a*, Frontal view. In the upper portion *O. alula* has overgrown *A. tincta*, whereas in the lower portion the colony which is growing fastest here will overgrow, that is, that colony which first produces a zooid here. *b*, side view, showing currents and interference effect.

interfering with the access of its competitor to food resources (Fig. 1*a*, *b*).

Onychocella alula (Hastings) and *Antropora tincta* (Hastings) are two encrusting anascan bryozoans which co-occur on intertidal and shallow subtidal cobble in the eastern Pacific of Panama. The feeding organs of individual zooids (lophophores) extend only a few hundred micrometres above the surface of the colony (*O. alula* and *A. tincta*, respectively: 17 and 12 tentacles, 790 and 410 μm tentacular bell diameter, 320 and 280 μm introvert length, 545 and 295 μm tentacle length), within or very close to the layer of stagnant water adjacent to the substratum[21,22]. The dominant characteristics of the flow regime surrounding them are those produced by the feeding activities of the organisms themselves[21,22]. Hence, when in contact, the feeding currents produced by the larger, more robust lophophores of *O. alula* might be expected to interfere with currents produced by adjacent smaller lophophores of *A. tincta* (Fig. 1*b*).

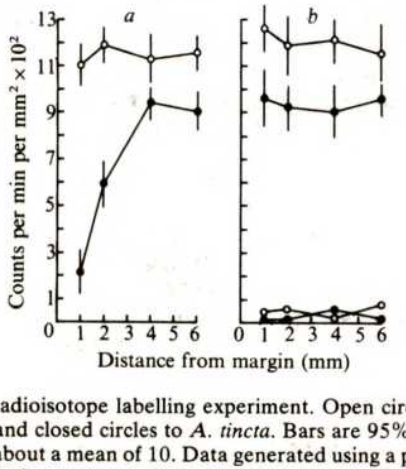

Fig. 2 Radioisotope labelling experiment. Open circles refer to *O. alula* and closed circles to *A. tincta*. Bars are 95% confidence intervals about a mean of 10. Data generated using a piece of lead shaped to the contour of the colonies and impermeable to γ emissions. *a*, Interaction experiment; *b*, bottom lines: rinsing process control; top lines: intracolony effects control.

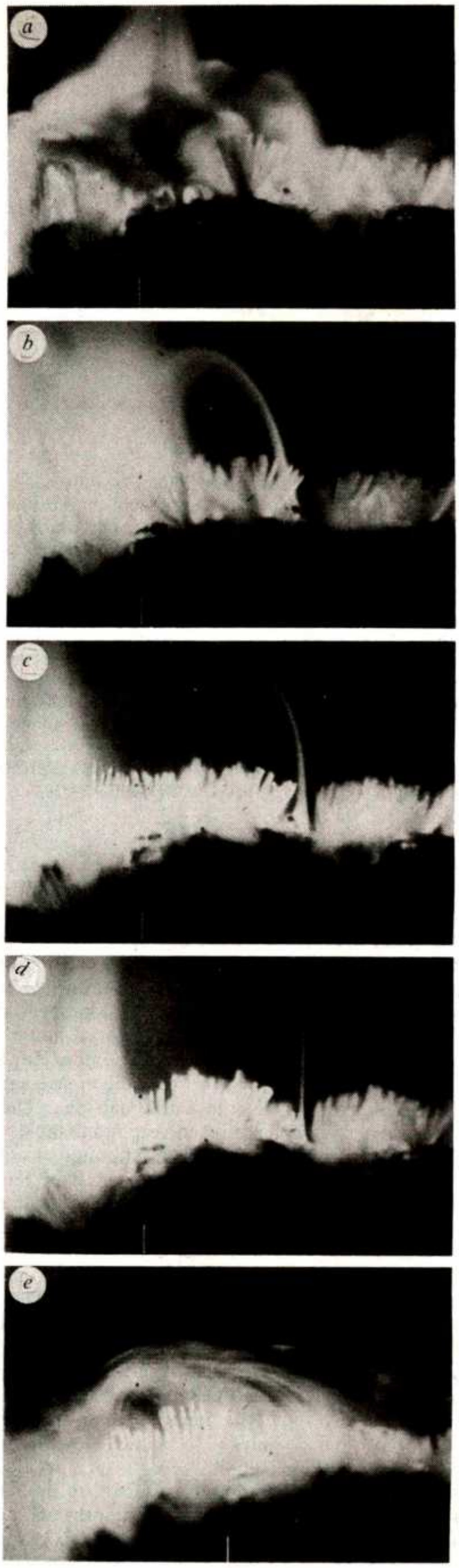

Fig. 3 Microcurrent interference. The point of colony contact is indicated by the bar at the base of all frames. *A. tincta* is the colony to the right of the bar. *a–d*, elapsed time: *a*, 0:00; *b*, 0:19; *c*, 0:45; *d*, 3:32 (min:s). *e*, elapsed time: 0:53. Magnification ≅ ×60. See text for discussion.

To test this hypothesis, competing colonies were allowed to feed on radioactive particles, and feeding efficiency (clearance rate) was calculated as a function of the distance from the interspecific contact. Substrata on which *O. alula* and *A. tincta* were in contact, but on which overgrowth had not yet occurred, were collected at Punta Paitilla, Panama, and maintained in running seawater at the Smithsonian Tropical Research Institute. Colonies were allowed to acclimate and when feeding, 5 ml of human albumin microspheres (3M Corp., size range 5–90 μm, $\bar{x} = 25$ μm, s.d. = ±15 μm, concentration = $5 \pm 2 \times 10^6$) labelled with 5 mCi of ^{99}Tc were introduced into the container. The experiment was aborted if feeding of both colonies was not continuous for the entire 1 h experimental period. Complexities due to possible translocation effects do not occur over this time interval[23]. On termination, colonies were killed, rinsed for 1 h under a high-speed jet of seawater, and counted using facilities at the Gorgas Memorial Hospital, Canal Zone. Results are as hypothesised (Fig. 2*a*); the smaller *A. tincta* showed a significant local reduction in clearance rate in contact with *O. alula*, but *O. alula* showed no such reduction. Two control experiments were also carried out. Dead colonies were allowed to 'feed' on labelled microspheres and only a negligible proportion of these remained adherent to colony surfaces after rinsing (Fig. 2*b*, bottom lines). *O. alula* and *A. tincta* were allowed to feed on labelled microspheres in the absence of one another to control for intracolony effects, but no such effect was detected (Fig. 2*b*, top lines).

The hypothesis that the clearance rate reduction is due to an active interference was confirmed by producing videotapes of interacting colonies. Using a hypodermic syringe, a thin stream of milk–seawater suspension[24] was introduced into an experimental container above the interactive margin. The stream was initially disrupted by feeding activity, forming a broad cone of milk (Fig. 3*a*), which then dispersed to form a cloud above the *O. alula* colony (Fig. 3*b*). Within seconds, the feeding of *O. alula* filtered the water sufficiently for a zone of cleared water, enclosed by the emerging white band (Fig. 3*b*, *c*), to form. This zone expanded and, after several minutes, stabilised over the *A. tincta* colony (Fig. 3*d*), as indicated by the stationary white band. In this region, *A. tincta* had access only to water previously filtered by *O. alula*. The width of this zone measured from the interspecific margin to the stationary band was 3.07 ± 0.24 mm, equivalent to the distance of a clearance rate reduction in the labelling experiment (2–4 mm, see Fig. 2*a*). In an additional experiment (Fig. 3*e*), where milk was added above the *A. tincta* colony, no zone of cleared water was produced. In contrast to the first experiment, the milk streams away from *A. tincta* lophophores to those of *O. alula*. In both cases, *O. alula* interfered with the access of *A. tincta* to food resources, that is, competition for food occurred between these suspension feeders.

To overgrow *A. tincta*, *O. alula* must grow faster than *A. tincta* at the point of colony contact. Unpublished field observations of colonies not in competition have shown that *A. tincta* grows faster than *O. alula*, irrespective of colony size. The observation that *O. alula* interferes with the access of *A. tincta* to food resources and that this interference effectively reduces *A. tincta* food intake suggests that this overgrowth is derived from the mechanism proposed. Competitive interactions for spatial resources may be mediated by competition for food.

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Action of a nematode-trapping fungus shows lectin-mediated host–microorganism interaction

Birgit Nordbring-Hertz

Department of Microbial Ecology, University of Lund, Ecology Building, Helgonavagen 5, s-223 62 Lund, Sweden

Bo Mattiasson

Biochemistry 2, Chemical Centre, University of Lund, S-22007 Lund, Sweden

Many nematode-trapping fungi capture nematodes using an adhesive present on specific capture organs (for review see ref. 1). Until recently, the mechanism of adhesion was completely unknown. In the case of *Arthrobotrys oligospora*, one of the most common nematophagous fungi, nematodes are trapped in three-dimensional structures of the adhesive network type (Fig. 1*a*). When a suspension of nematodes is added to an agar culture of the fungus, nematodes are immediately captured and firmly held by the traps. The nematode cuticle is lysed at the point of contact and penetrated by a hypha within one hour[2]. An increased secretion by the fungus of a mucilaginous substance in the presence of prey has been shown by scanning and transmission electron microscopy[2,3]. Our hypothesis is that the firmness of attachment to the traps despite the struggle of the nematode is due to a series of events, beginning with an interaction between complementary molecular configurations on the nematode and fungal surfaces. We show evidence here for the presence of a lectin on the traps of *A. oligospora* which binds to a carbohydrate on the nematode surface.

Developmentally regulated lectins have been identified in cellular slime moulds, and lectin activity is closely correlated with the development of cohesiveness between the cells[4–6]. Fungal lectins are produced in the symbiotic relationship between algae and fungi in lichens[7]. The role of lectins in other host-microorganism interrelationships, for example *Rhizobium*-leguminous plant associations, is well documented[8–11].

If a lectin-carbohydrate interaction is involved in this relationship, then pre-exposure of the lectin-carrying structure to specific carbohydrates would prevent attachment of the nematodes to the traps. As capture usually takes place within 30 min, microscopic observations at intervals during a 24-h period would give good information on the inhibitory effects of carbohydrates.

To investigate the nematode-trapping ability of *A. oligospora* Fres. (ATCC 24927), the fungus was grown on dialysis membrane on an agar surface[12]. The membranes were transferred to empty Petri dishes before capture of nematodes was tested to eliminate the effects of agar. To test inhibition of capture, cultures of the fungus were flooded with carbohydrate solutions for 24 h before addition of a water suspension of nematodes. Axenically cultivated bacteria-feeding nematodes (*Panagrellus redivivus* Goodey) were used throughout[3]. Alternatively, nematodes were suspended for 3–24 h in the same carbohydrate solutions, washed and added to untreated fungal cultures.

Flooding of the fungal colony with 20 mM *N*-acetylgalactosamine (GalNAc) resulted in total inhibition of capture over a 24-h period (Table 1). At this concentration capture was delayed by treatment with melibiose, whereas nematode-trapping ability was unaffected by all other carbohydrates. At higher concentrations of carbohydrates (200 mM) a less pronounced specificity was observed, as maltose, galactose and related sugars prevented capture. These results indicate low specificity of the lectin when exposed to high concentrations of carbohydrate and agree well with reports on the lectins from *Dictyostelium discoideum*[4,6]. In controls flooded with water or phosphate-buffered saline (0.15 M NaCl, 0.01 M potassium phosphate buffer, *p*H 7.2, PBS) total capture on dialysis membranes occurred within 30 min–3 h. Conversely, treatment of the nematodes with carbohydrates at 20 and 200 mM failed to inhibit capture. Therefore, the possibility that the carbohydrate-binding activity was located on the nematode was excluded.

The fact that nematode-trapping ability could be suppressed by preincubating the fungus with specific carbohydrates led to the conclusion that a lectin on the fungus is involved in, or responsible for, the interaction. A further consequence of this is that the surface of the nematode must offer suitable binding sites for the lectin, that is GalNAc groups must be present. Two series of experiments tested these hypotheses.

To see if the proposed trap lectin binds to a model prey, the fungus was grown on a piece of dialysis membrane and exposed to an excess of red blood cells (RBC). RBC from different blood groups carry different polysaccharides on their surfaces. Blood group A is characterised by a terminal GalNAc, B carries galactose and blood group O fucose. After incubation for 3–4 h at room temperature the membrane strips were carefully washed in PBS and observed under the microscope.

Significant attachment of RBC took place only at the capture organs (Fig. 1*b*). There was no marked difference between the blood groups, although group A showed a tendency to attach more easily. Thus the proposed trap lectin did not show strict specificity for GalNAc in these *in vivo* tests. As both galactose and fucose had some inhibitory effect on capture of nematodes (Table 1) this low specificity is expected. The developmentally regulated lectins isolated from *D. discoideum* showed a similar low specificity[4,6], and various degrees of specificity are reported for other lectins[13].

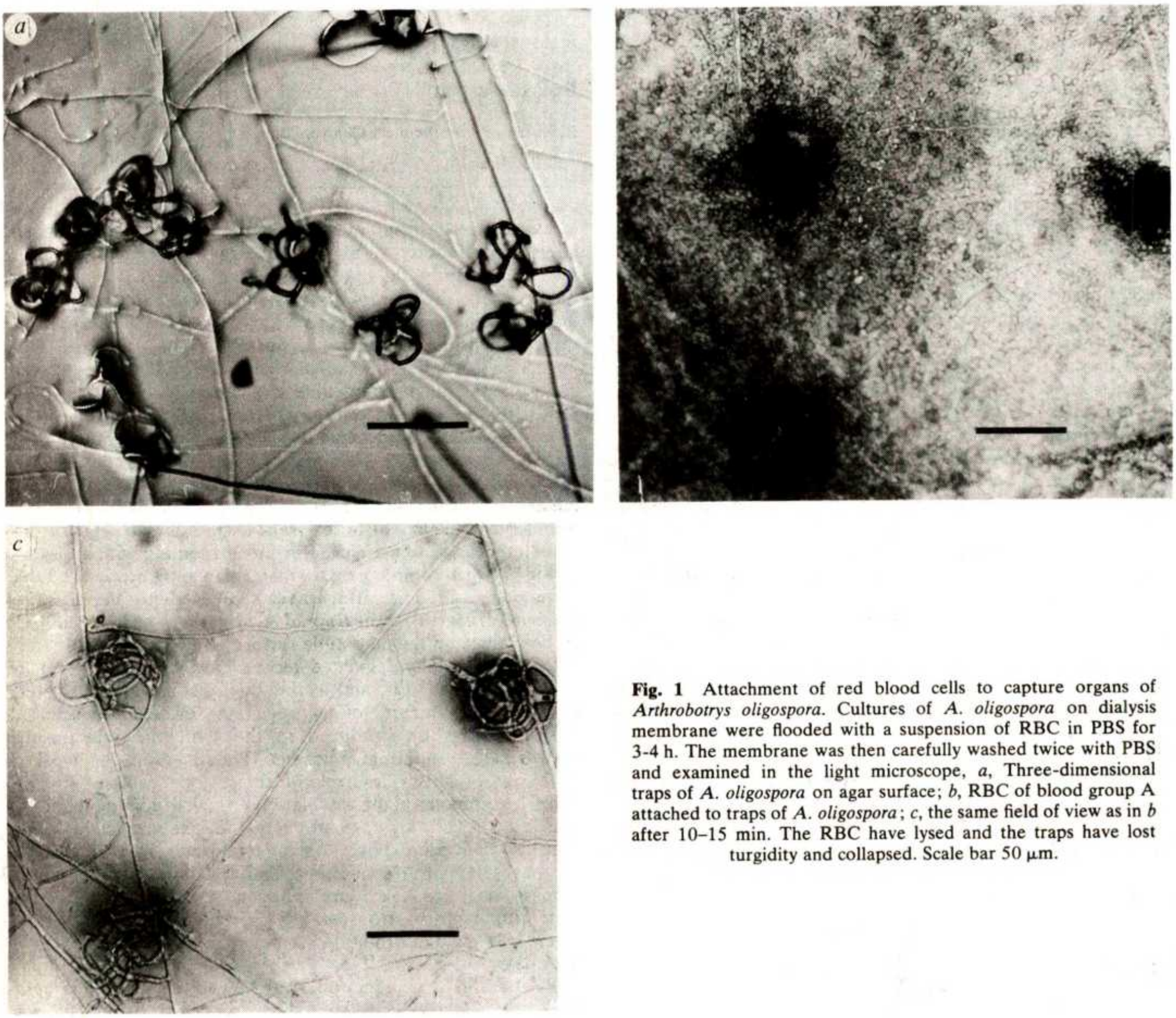

Fig. 1 Attachment of red blood cells to capture organs of *Arthrobotrys oligospora*. Cultures of *A. oligospora* on dialysis membrane were flooded with a suspension of RBC in PBS for 3-4 h. The membrane was then carefully washed twice with PBS and examined in the light microscope, *a*, Three-dimensional traps of *A. oligospora* on agar surface; *b*, RBC of blood group A attached to traps of *A. oligospora*; *c*, the same field of view as in *b* after 10–15 min. The RBC have lysed and the traps have lost turgidity and collapsed. Scale bar 50 μm.

Table 1 Effect of saccharides on the nematode-trapping ability of *Arthrobotrys oligospora*

Saccharide	Trapping ability at: 20 mM	200 mM
N-acetyl-D-galactosamine	–	ND
α-D-galactose	+	–
2-deoxy-D-galactose	+	–
fucose (6-deoxy-L-galactose)	+	(–)
N-acetyl-D-glucosamine	+	ND
α-D-glucose	+	+
2-deoxy-D-glucose	+	+
D(+)-mannose	+	+
α-methyl-D-mannoside	+	+
L-sorbose	+	–
D-fructose	+	+
L-xylose	+	+
D-arabinose	+	–
α-D-melibiose	(–)	–
lactose	+	–
maltose	+	–
D(+)-trehalose	+	+
sucrose	+	+

* The fungus was grown on strips of dialysis membrane[12] placed on the surface of a low-nutrient mineral salts medium[18]. Trap formation was induced by addition of a small amount of nematodes to 3–4 d old cultures. Trap-containing strips (7–14 d) were transferred to empty Petri dishes and flooded for at least 20–24 h with the appropriate carbohydrate (20 or 200 mM solution in distilled water or in phosphate-buffered saline). After removal of excess carbohydrate, 1–2 drops of a nematode suspension in water (approx. 1,000 nematodes ml^{-1}) were added to the fungal culture to test capture ability. Microscopic observations of capture were made after 30 min, 3 h and about 24 h. Results of 2–10 different experiments for each carbohydrate. +, normal capture; –, inhibition of capture; (–), capture delayed. ND, not determined.

It was observed that RBC, accumulated around the traps (Fig. 1*b*), lysed within 1 h. As lysis of RBC occurred to only a small extent spontaneously during these experiments, the effect was attributed to the trap. Immediately after RBC lysis the traps lost their three-dimensional appearance and collapsed. RBC stabilised by crosslinking with glutaraldehyde (80 μM for 6 h at about 20 °C) were captured by the traps in the same way as untreated cells but did not lyse.

It was not possible to determine the number of RBC bound per unit area or unit weight of fungal biomass in these *in vivo* tests. This was due to differences in trap size and distribution on the dialysis membranes between the experiments and to the irregular shape of the trap structures.

It seems that binding is achieved primarily by the GalNAc structure as inhibition of capture at the lower concentration of carbohydrate occurred only with GalNAc. To demonstrate GalNAc residues on the nematode surface, nematodes were exposed to a peroxidase–lectin conjugate prepared[14] with lectin from *Helix pomatia* with affinity for GalNAc[15]. As a blank, the nematodes were exposed to free peroxidase and otherwise treated identically. Incubation of nematodes in substrate (14 mM phenol, 0.8 mM 4-aminoantipyrine, 2 mM hydrogen peroxide) and assay for peroxidase activity[16] according to procedures developed for assay of particle-bound enzymes[17] showed that binding had taken place. Unspecific adsorption of free peroxidase to the nematode or inherent enzyme activity gave some background activity. However, peroxidase activity of conjugate-treated nematodes was approximately six times that of the peroxidase-treated controls. This was interpreted as indicating the presence of GalNAc residues on the nematode surface. Furthermore, on exposing the nematodes to the enzyme–lectin conjugate in the presence of free GalNAc, no activity above the background was observed.

We conclude that a developmentally regulated lectin is present on the traps of *A. oligospora* and binds to carbohydrates on the nematode surface as well as to RBC. The binding initiates an enzymatic alteration of both nematode and erythrocyte surfaces leading to penetration of the nematode cuticle by a hypha[2] and lysis of the RBC. The results contribute a new approach to the solution of the problem of specificity in this host–microorganism interrelationship.

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N-acetyltransferase activity responds to environmental lighting in the eye as well as in the pineal gland

Sue Binkley, Michael Hryshchyshyn & Kathleen Reilly

Biology Department, Temple University, Philadelphia,
Pennsylvania 19122

N-acetyltransferase (NAT), which converts serotonin to N-acetylserotonin, is widely distributed in the vertebrate body[1,2]. In the pineal gland, however, NAT activity shows circadian changes which respond to environmental lighting both *in vivo* and *in vitro*[3,4]. NAT is responsible for rhythmic production of the pineal hormone melatonin, which functions in body colour changes[6], photoperiodic control of reproduction[7,8], and behavioural circadian rhythms[9,10]. The rhythmic changes in NAT have so far been thought unique to the pineal gland. Here we report data for ocular NAT showing a change in a daily light–dark cycle and modification by environmental lighting. The finding of light modified NAT in the eye is significant because it may explain the failure of some investigators[11] to find effects of pinealectomy and it may also explain the occurrence of rhythmic melatonin in the blood of some species after pinealectomy[12–14]. The prerequisites for the production of melatonin exist in the eye as well as in the pineal gland: serotonin[15], hydroxyindole-*O*-methyltransferase activity[16] and *N*-acetylindole[17] are present. Furthermore, labelled serotonin is converted to melatonin by the retina *in vitro*[18]. Our finding of NAT and its modification by light and dark completes the argument for ocular capacity for melatonin production in a cyclic manner modified by environmental lighting.

In our experiments the eyes and the pineal gland were investigated in parallel by assaying both structures from animals during light–dark treatments. The data in Fig. 1 (and in two to four replicate experiments) illustrate the following statistically significant results ($P < 0.05$): (1) In a light–dark cycle, dark-time NAT was 7.0–11.8 times as high as light-time NAT in the pineal gland; dark-time NAT was 2.7–2.9 times light-time NAT in the eye. (2) When chicks were placed in constant dark (DD), pineal NAT in their subjective dark-time was 3.7–5.9 times that in the

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Table 1 Ocular NAT in four species was measured in the light and dark times of a light–dark cycle (LD12:12)

	N-acetyltransferase activity (nmol per eye per h) Light	Dark
Chick, *Gallus domesticus*	3.038 ± 0.122	8.726 ± 0.898
Rat, *Rattus norvegicus*	0.475 ± 0.066	1.322 ± 0.148
Sparrow, *Passer domesticus*	0.119 ± 0.029	0.483 ± 0.053
Hamster, *Mesocricetus auratus*	0.726 ± 0.148	0.527 ± 0.132

Values are means ±s.e.m., $N = 4$.

light-time; in contrast, in the eye, subjective dark-time NAT was not significantly different from subjective light-time NAT. (3) When the chicks were placed in constant light (LL) subjective pineal dark-time NAT was suppressed (to 25–27% of its LD value); ocular dark-time NAT was likewise suppressed (to 35–38% of its LD value). (4) NAT shows a 'rapid response' to light during the dark-time[19,20]. Chicks were exposed to light for 1 h during their subjective dark-time which suppressed dark-time pineal NAT (61–76%) and also suppressed ocular NAT (36–59%).

The specific activity of NAT in the pineal gland was up to three times that in one eye during the dark-time. Table 1 shows data for four species kept in light–dark cycles. The chick, the sparrow, and the rat all had ocular dark-time NAT three to four times higher than ocular light-time NAT. The data for ocular NAT and its modulation by environmental lighting are convincing evidence that the pineal gland is not singular in its ability to metabolise serotonin and produce melatonin in a rhythmic fashion.

In the experiments reported here—two-point experiments—there was no evidence that the ocular NAT has a persistent circadian rhythm in the same conditions where such a rhythm clearly occurs in the pineal gland. Nor did the eye of the hamster have an NAT rhythm in the LD cycle. However, these two-point experiments are not conclusive evidence that there is no circadian rhythm—it is possible to miss a narrow peak of activity even with more frequent time points.

When NAT activity in the two eyes was compared the values were positively correlated ($r = 0.82$ calculated for six pairs from LD chicks). In the experiments detailed in Fig. 1 and Table 1, NAT was measured in the neural retina plus the pigmented layer plus the accompanying wetting vitreous humor. In a separate experiment done to partition the NAT activity (Table 2), 41–56% of NAT was localised in the neural retina and 42–57% was in the pigmented layer; only 2% was in the 10-μl sample of vitreous humor (used to estimate the accompanying wetting of the neural retina and pigmented layers by humor during the dissection). As whole eyes were not analysed, the measurements do not represent the total ocular melatonin synthesising capacity. Bubenik *et al.*[17] localised *N*-acetylindole to the outer layer of the neural retina.

To make physiological sense of the relative contributions of pineal and ocular melatonin requires knowledge of the target(s) and functions of melatonin. Melatonin synthesis by the eye could be an endocrine function, or, alternatively, the eye itself may be the target. For endocrine function, the eye may be an additional source of melatonin which acts in the circulation as a hormone augmenting pineal production.

Table 2 NAT was measured separately in the three portions of the eye which were used together for the experiments in Fig. 1 and Table 1

	N-acetyltransferase activity Light	Dark
Eye		
Vitreous humor	0.070 ± 0.027	0.144 ± 0.065
Neural retina	1.654 ± 0.270	9.678 ± 1.157
Pigmented layer	2.304 ± 0.350	7.409 ± 0.777
Pineal gland	2.632 ± 0.451	22.099 ± 4.374

Each value is a mean ($N = 4$) ±s.e.m. NAT is given as nmol per tissue per h for the neural retina and pigmental layer (one eye), as nmol per gland per h for the pineal gland, and as nmol per 10μl per h for the vitreous humor.

There are several hypothetical roles for melatonin within the eye itself. Ocular melatonin may function in the daily rhythms reported for outer disk shedding in the retina[21]. Two types of movement have been noted in the eyes of lower vertebrates and some birds: retinal cones elongate and shorten[22], and pigment migration is thought to occur in the melanin containing cells[23]. The melanin pigment layer widens in the dark and narrows in the light—pinealectomy abolishes the dark displacement of pigment, melatonin increases the displacement[23]. The cones shorten in the light and lengthen in the dark—short-term effects which are superimposed on an underlying circadian rhythm which persists in constant light or constant dark[22,24].

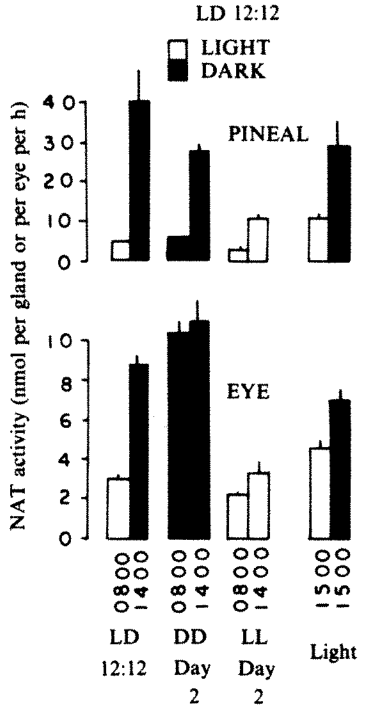

Fig. 1 NAT activity in the eyes and pineal glands of chicks kept in a light–dark cycle (LD), constant dark (DD) or constant light (LL). Chicks were kept in a light–dark cycle, LD12 h : 12 h, lights-off 1000, from day 1 after hatching until they were 23 days of age. The chicks were then divided into groups and kept for 2 more days in either the same LD cycle, DD or LL. On the second day they were killed at 0800 (subjective day-time) or at 1400 (subjective night-time). A fourth group of chicks were kept in the LD cycle but exposed to light for one hour (1400–1500) during their subjective dark-time—this group and controls kept in the dark were killed at 1500. The data shown here are from one of two replicate experiments ($N = 4$ chicks per group, bars represent mean values ±s.e.m.); the LD experiment was performed four times. For these experiments the combined neural retina plus pigmented layer was dissected from the eyes immediately after the chicks were killed. The ocular tissue and pineal glands were then quick frozen on dry ice and assayed for NAT within a week[5].

We conclude that the eye is capable of rhythmic melatonin synthesis (in the chick, sparrow and rat), and that the chick ocular NAT is modified by environmental lighting (constant light, dark-to-light transitions). It is probable that ocular melatonin gets into the bloodstream, because some pinealectomised animals have rhythmic melatonin circulating after pinealectomy[12–14].

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Note added in proof: Hamm and Menaker (personal communication) have evidence that the ocular NAT rhythm is circadian with a later and narrower peak on the second day of constant dark; we now have evidence for circadian clock involvement from light-to-dark transition experiments.

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Modulation of juvenile hormone synthesis by an analogue in the cockroach

S. S. Tobe

Department of Zoology, University of Toronto, Toronto, Ontario, Canada M5S 1A1

B. Stay

Department of Zoology, University of Iowa, Iowa City, Iowa 52242

The juvenile hormones (JH) are novel insect sesquiterpenoid products synthesised by paired endocrine glands known as the corpora allata (CA). These glands are innervated by neural and neurosecretory elements originating in the central nervous system (CNS)[1–3] and are known to undergo predictable changes in synthesis and release of JH during the reproductive cycle[4–8]. This implies that the CA are regulated by intrinsic physiological signals. For example, in the viviparous cockroach, *Diploptera punctata*, if the haemolymph titres of JH are experimentally altered by removal of one member of the pair or by implantation of additional CA, the original CA undergo appropriate corrective responses in JH synthesis[9,10]. This is partly due to signals from the CNS because denervation of the CA attenuates the compensatory change[10]. The depression in JH synthesis after addition of exogenous JH to the system has also been observed[11,12]. However, there have been no direct measurements of JH synthesis following treatment with exogenous JH analogues *in vivo*. We show here that in *Diploptera punctata*, topical application of a JH analogue (JHA) depresses the synthesis of JH at high doses but stimulates synthesis at low doses and that the analogue can support oocyte growth and maturation in the absence of the CA and that application to intact animals stimulates oocyte growth rates. These results indicate that the analogue can replace authentic JH.

Mated females of *Diploptera punctata* were allatectomised between 1 and 5 h after adult ecdysis[9]. Three to four days later, the JH analogue ZR512 (ethyl (2*E*, 4*E*)-3,7,11-trimethyl-2,4-dodecadienoate, Zoecon) was applied in 5 μl of acetone to the dorsal surface of the abdomen (wings were removed). The mean weight of these females was 190±3 mg. Figure 1 shows a semi-logarithmic plot of ZR512 dose against the basal oocyte length 6 days after analogue application. At 2.5 μg the oocyte response was slightly greater than that of the controls but not significantly different; between 10 and 100 μg it was virtually linear; at 200 μg the oocytes attained maximum mature length (1.65 mm) with 80% chorionated, ready for oviposition. Oviposition was elicited in 6 of the 7 females treated with 200 μg and in 5 of the 9 females receiving 100 μg ZR512. None of the females treated with acetone ($n = 9$), 25 μg ($n = 10$), or 10 μg ($n = 5$) ZR512 oviposited. From these observations we conclude that a single topical application of ZR512 (≥ 100 μg) in acetone effectively mimics the action of JH in *Diploptera punctata*, permitting both oocyte maturation and oviposition in the absence of authentic hormone.

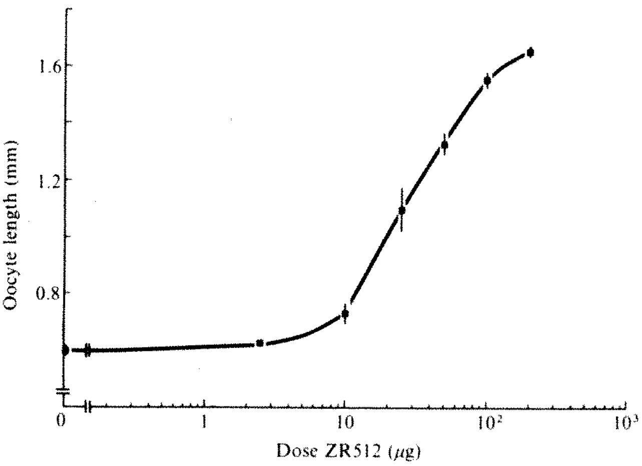

Fig. 1 Basal oocyte length on day 6 after treatment of allatectomised female *D. punctata* with ZR512. Oocytes attained mature length with the 200-μg dose. Each point represents the mean ± s.e.m. of determinations on 5–10 animals.

To test the effects of this analogue on the rate of JH synthesis by the CA, normal mated females were treated with various doses of ZR512 between 1 and 5 h after adult emergence. Every day for six days after the treatment, the length of the basal oocytes was recorded and the rate of C_{16}JH (JH III) synthesis by the CA was assayed[4,5]. Figure 2*a* shows the progress of egg growth for 100, 25 and 2.5-μg doses of ZR512, and acetone-treated controls. The rate of oocyte growth showed a positive correlation with the concentration of ZR512 applied. The difference between oocyte lengths in 25 μg- and 100 μg-treated animals and controls is significant for days 2–6 ($P < 0.001$; Students *t*-test), whereas in animals treated with 2.5 μg, although the oocytes were larger than controls on days 2–5, only on day 4 is the difference significant ($0.02 < P < 0.01$). These results indicate that exogenously applied analogue may act with JH released by the CA to raise the titre of JH–JHA such that eggs grow more rapidly in treated animals than in controls. (For example, the control females had 1.33 mm-long oocytes on day 5 whereas this length was achieved at 4.25, 3.25 and 2.75 days in females treated with 2.5, 25 and 100 μg of analogue respectively.) Alternatively, the ZR512 may stimulate the CA to synthesise JH at a greater rate, causing an elevation in JH titre.

The choice between these hypotheses is facilitated by the *in vitro* determination of the rate of JH synthesis by the CA of the same animals for which egg growth is shown (Fig. 2*a*). These measurements provide an estimation of the contribution of endogenous JH towards the increased rate of oocyte growth (Fig. 2*b*). At 100 μg ZR512, the normal cycle of JH synthesis was depressed, reaching a maximum rate of only 28 pmol per h per pair on day 3 and minima on days 2 and 5 of 6–7 pmol per h per pair. Animals receiving 25 μg of ZR512 showed rates of JH synthesis similar to those of acetone controls on days 2–4 but depressed rates thereafter (maximum rate of JH synthesis 54 pmol per h per pair). Maximal rates of JH synthesis (87 pmol

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per h per pair) by CA of control animals were observed on day 6. On the basis of oocyte lengths attained by this time (Fig. 2*a*) and from control measurements reported elsewhere[9,10], this value probably represents the peak of the normal cycle. In sharp contrast to the depression of JH synthesis by 100 and 25 µg doses of ZR512, animals receiving 2.5 µg of ZR512 showed a marked stimulation in JH synthesis on day 4 (126 against 49 pmol per h per pair) ($P = 0.028$). Similar stimulation on day 4 has been observed after treatment with 2.5 µg C_{16} JH (unpublished data). We conclude that the high doses of ZR512 depress synthesis of JH, whereas the low dose stimulates synthesis. The accelerated oocyte growth observed at high doses of ZR512 seems to be due to the cumulative action of the analogue and endogenous JH on the oocytes causing an elevation in the titre of JH-active compounds.

To account for these opposing actions of the applied hormone analogue, we hypothesise that below a certain dose, JH synthesis is stimulated by the analogue, (that is, shows positive feedback) and that at a higher dose, a negative feedback operates to depress JH synthesis. Such a hypothesis would also account for the increase in rates of JH synthesis after unilateral allatectomy and the suppression of JH synthesis in the presence of supernumerary CA[9,10] and for the rapid changes in JH synthesis during the normal reproductive cycle[5]. For example, a positive feedback loop may be responsible for the rapid increase in JH synthesis observed in normal females between days 3 and 5 after emergence[5]. Such loops have been proposed for insect endocrine systems, particularly for the interaction between the CA and cerebral neurosecretory cells[13–16] and between the prothoracic glands and cerebral neurosecretory cells[17–19]. However, definitive proof for such pathways in insect systems has been lacking, although they are well known in vertebrate systems—for example the preovulatory surge of luteinising hormone which occurs as a result of increasing titres of estradiol[20]. It is significant that in *D. punctata* the proposed positive feedback loop responsible for the stimulation of JH synthesis in animals treated with 2.5 µg ZR512 operates only for a short period during the reproductive cycle, with a maximum effect on day 4. This is precisely the time at which rates of JH synthesis are rapidly increasing in control animals[5]. This suggests the existence of a temporal gate in the centre which regulates the CA—thus the regulatory centre may be sensitive or responsive to increasing titres of JHA only for a brief period during the reproductive cycle.

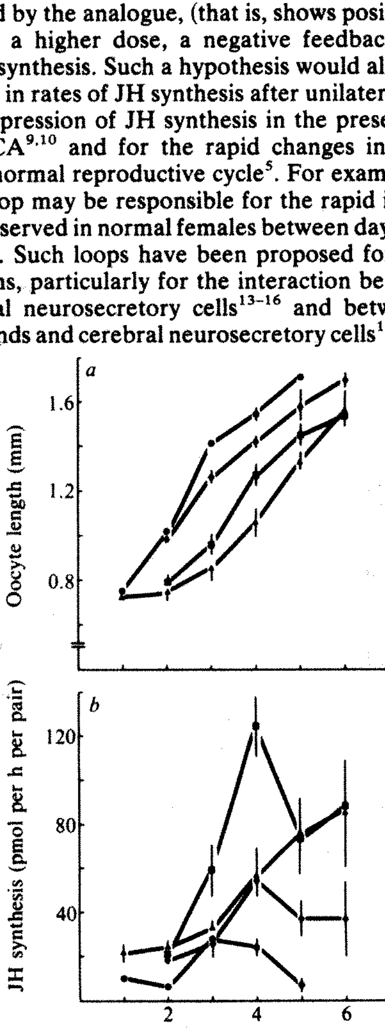

Fig. 2 The effect of ZR512 on oocyte growth and JH synthesis in *D. punctata*. Animals were treated topically on day 0 and assayed on days 1–6. *a*, Mean oocyte lengths ±s.e.m.; *b*, mean rates of JH synthesis ±s.e.m. $n = 5$, except for day 4 where $n = 10$ and day 5 where $n = 10$ for 2.5 µg, $n = 9$ for 25 µg and acetone and $n = 7$ for 100 µg. Rates of synthesis of C_{16}JH were determined using an *in vitro* radiochemical assay which uses the incorporation of the methyl moiety of [^{14}C-methyl]methionine into C_{16}JH. Individual pairs of CA were incubated for 3 h at 28 °C in medium 199 (Hanks' salts; with glutamine, HEPES buffer, 25 mM, *p*H 7.2-GIBCO) plus Ficoll (20 mg ml^{-1}) to which [^{14}C-methyl]methionine (Amersham-Searle, final specific activity 35–39 mCi mmol^{-1}) was added. Procedures for the preparation of the incubation medium, and the separation and quantitation of biosynthesised radiolabelled JH have been described previously[4–5]. ●, 100 µg 2R512; ◆, 25 µg 2R512; ■, 2.5 µg 2R512; ▲, acetone.

These results also demonstrate that high doses of JHA suppress JH synthesis by the CA. Implantation of supernumerary CA[9,10], as a source of JH, as well as topical treatment with C_{16}JH (unpublished data) also result in a reduction in JH synthesis. The simplest explanation for this suppression is a negative feedback loop. Such mechanisms are well known in the regulation of endocrine gland function.

These feedback signals may operate on an intermediate regulatory centre outside the CA but it is conceivable that the signals may also feed back directly on the CA. However, Pratt and Finney[21] did not observe inhibition of JH synthesis by C_{16} JH *in vitro* in *Periplaneta americana*. On the other hand, we have shown that isolation from the regulatory centres of the CNS by denervation of the CA attenuates but does not totally abolish corrective responses by the CA[9,10] and, although this may suggest a feedback loop operating directly on the CA, it is equally likely that a regulatory centre outside the CA may be controlling the glands via a humoral pathway.

Although the mode of action of ZR512 still remains to be defined precisely, it is clear that, at high doses, it can be regarded as a suppressor of JH synthesis. Using the *in vivo–in vitro* assay described here, it is now possible to screen JH analogues for such activity. Those analogues which suppress CA activity but have no gonadotrophic activity may have potential as insect control agents. The only compounds known to have such activity are the precocenes[22,23] but their mode of action is unclear.

Our assay differs from other screening procedures[21–24] in that it permits a simultaneous assessment of analogues as inhibitors of both JH synthesis and ovarian growth in the same insect. The techniques described here should also be valuable for investigating the role of the centres outside the CA in regulating these endocrine glands.

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FSH stimulates hyaluronic acid synthesis by oocyte–cumulus cell complexes from mouse preovulatory follicles

John J. Eppig

The Jackson Laboratory, Bar Harbor, Maine 04609

A surge in the secretion of gonadotropins stimulates several processes in mammalian preovulatory follicles, including oocyte maturation, cumulus expansion (mucification) and ovulation. This surge results in increased circulating levels of both luteinising hormome (LH) and follicle-stimulating hormone (FSH). It has generally been thought that LH is primarily responsible for the stimulation of the maturational processes in preovulatory follicles that results in ovulation and corpus luteum formation, and that FSH stimulates the development of the next wave of preovulatory follicles. A direct role for FSH in preovulatory events has been unclear. However, two studies have suggested that FSH is essential for certain preovulatory processes. First, there is evidence that the production of plasminogen activator by granulosa cells is more sensitive to FSH than to LH[1]. Plasminogen activator could convert follicular fluid-borne plasminogen to plasmin, a protease capable of weakening the follicle wall[2]. Second, highly purified FSH, but not highly purified LH, stimulates cumulus expansion by isolated mouse oocyte–cumulus cell complexes[3]. A critical step in cumulus expansion is the deposition of a hyaluronic acid matrix, so that the synthesis of this glycosaminoglycan is very important in the preparation of the mouse oocyte–cumulus cell complex for normal ovulation. I report here that the synthesis of hyaluronic acid *in vitro* is stimulated by FSH but not by LH.

Biological grade preparations of FSH and LH stimulated hyaluronic acid synthesis by isolated mouse oocyte–cumulus cell complexes in a dose-dependent fashion (Fig. 1*a*). However, the complexes were much more sensitive to FSH than to LH, suggesting that the response to biological grade LH was due to contamination with FSH. To test this idea, the complexes were incubated in highly purified rat FSH and LH. The former, but not latter, significantly ($P < 0.01$) stimulated hyaluronic acid synthesis (Fig. 1*b*). Similarly, highly purified human chorionic gonadotropin (HCG), which competes for the same binding sites as LH[4], did not stimulate hyaluronic acid synthesis (Fig. 1*b*). As crude HCG significantly ($P < 0.01$) stimulated the synthesis of hyaluronic acid (Fig. 1*b*), this preparation is probably contaminated with chorionic FSH. Treatment of FSH-stimulated complexes with *Streptomyces* hyaluronidase indicated that 90% of the ^{3}H-glucosamine incorporated into cetylpyridinium chloride-precipitable counts was incorporated specifically into hyaluronic acid (Fig. 1*b*). Dibutyryl cyclic AMP, theophylline and cholera toxin all significantly ($P < 0.01$) stimulated hyaluronic acid synthesis (Fig. 1*c*).

It is concluded that FSH, and not LH, stimulates the synthesis and deposition of hyaluronic acid by isolated mouse oocyte–cumulus cell complexes. Therefore, FSH plays a direct, critical role in an important event, hyaluronic acid synthesis, that is essential for the cumulus expansion preceding normal ovulation. This is the first demonstration of a specific biosynthetic process, related to the ovulatory process, that is stimulated directly by FSH and cannot be directly stimulated by LH. The finding that dibutyryl cyclic AMP, theophylline (an inhibitor of cyclic AMP degradation) and cholera toxin (a stimulator of cyclic AMP synthesis) all stimulated hyaluronic acid synthesis suggests that the action of FSH is probably mediated by cyclic AMP.

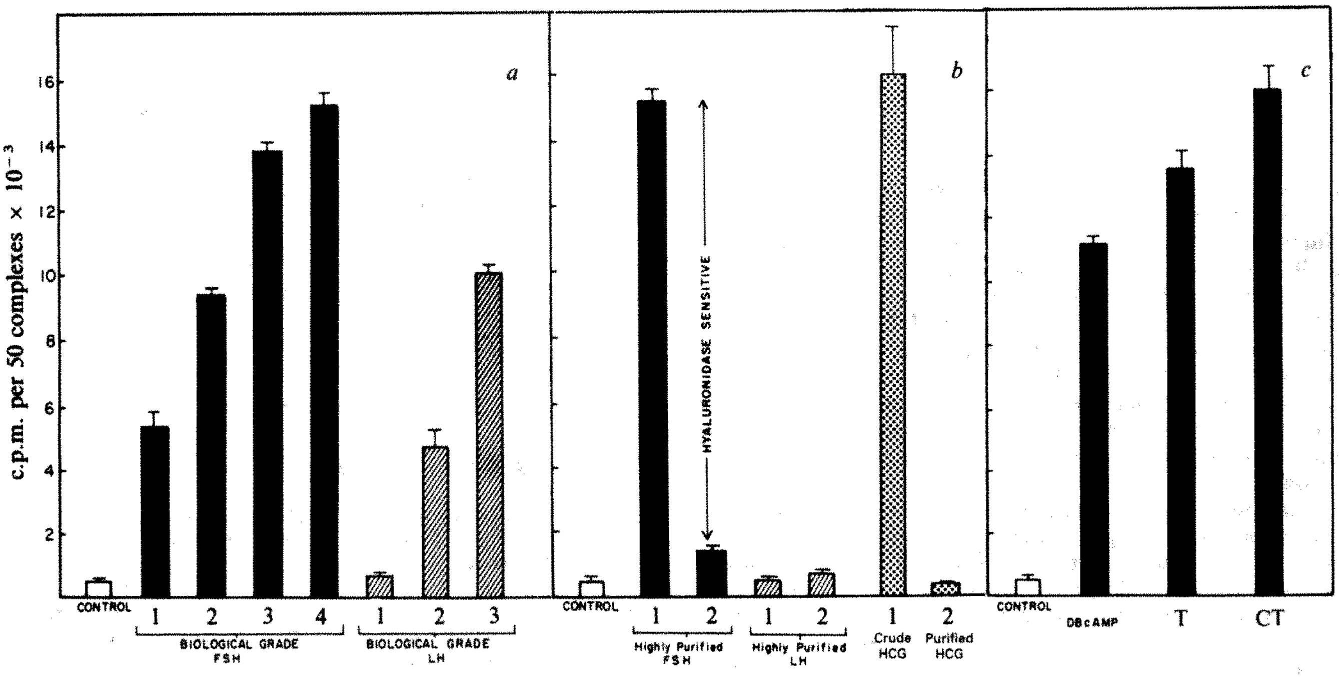

Fig. 1 (C57BL/6J × LT/Sv)F$_1$ mice, 26–28-d-old, were injected with 5 IU of pregnant mare serum gonadotropin. Forty-eight hours later the large graafian follicles were punctured with 25-gauge needles and the oocyte–cumulus cell complexes were removed and washed by serial passage, using micropipettes, through four dishes containing 2.0 ml of Whitten's medium[6] supplemented with 5% fetal bovine serum (Sterile Systems Inc.). Hormones and isotope were added to a culture dish containing 2.0 ml of medium and incubated for 17 h at 37 °C in an atmosphere of 5% O_2, 5% CO_2 and 90% N_2. The following hormones were added at various concentrations: *a*, biological grade FSH (NIAMDD-rat FSH-B1), 0.1 μg ml^{-1}(1), 0.5 μg ml^{-1}(2), 1.0 μg ml^{-1}(3), 5.0 μg ml^{-1}(4); biological grade LH (NIAMDD-bovine LH-B10), 1.0 μg ml^{-1}(1), 5.0 μg ml^{-1}(2), 10.0 μg ml^{-1}(3); *b*, highly purified FSH (NIAMDD-rat FSH-I-4), 1 μg ml^{-1}(1, 2); highly purified rat LH (NIAMDD-rat LH-I-4), 1 μg ml^{-1}(1), 5 μg ml^{-1}(2); crude HCG (Sigma), 5 IU ml^{-1}; highly purified HCG (R. E. Canfield preparation CR-119), 5 IU ml^{-1}; *c*, dibutyryl cyclic AMP, 5 mM (DBcAMP, Sigma); theophylline 5 mM(T, Sigma); cholera toxin 2 μg ml^{-1}(CT, Sigma). Hyaluronic acid synthesis was measured exactly as described by Solursh[7], in groups of 50 oocyte–cumulus cell complexes incubated in medium containing 25 μCi ml^{-1} of D-[6-^{3}H]glucosamine hydrochloride, specific activity 38 Ci mmol^{-1} (Amersham) for 17 h. Specificity of isotope incorporation into hyaluronic acid was determined by sensitivity of the labelled material to highly specific *Streptomyces* hyaluronidase[8]. After the 17-h labelling period some groups of the washed complexes were incubated in 5 IU ml^{-1} *Streptomyces* hyaluronidase (Calbiochem) at 37 °C for 3 h and then assayed according to Solursh[7]. At least three groups of 50 complexes were assayed for each group. Data are expressed as c.p.m. per 50 complexes ± s.e.m., and were subjected to one-way analysis of variance followed by the Student–Newman–Keuls multiple range test.

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Although LH is ineffective in stimulating hyaluronic acid synthesis *in vitro*, an indirect role for LH in regulating cumulus expansion *in vivo* cannot be excluded. The follicular fluid of preovulatory follicles contains significant amounts of FSH before the LH surge[5], but this FSH does not stimulate cumulus expansion before the gonadotropin surge. Perhaps LH affects the preovulatory follicle in some way that permits the oocyte–cumulus cell complex to respond to FSH and synthesise hyaluronic acid.

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Unmasking of fetal determinants on adult bone marrow cells

C. H. Granatek*

Department of Development Therapeutics

B. M. Scheinberg & P. M. Corry

Department of Physics, The University of Texas System Cancer Center,
M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77030

The phasing-out of an SV40 cross-reactive fetal antigen in the developing hamster fetus after day 10 occurs concomitantly with an increase of bound sialic acid in membrane, microsomal and soluble cell fractions[1]. A masking phenomenon is suggested by the fact that the antigen can be uncovered on older fetal tissue by trypsinisation[2]. Similarly, a murine oncofetal antigen under study in our laboratory is optimally expressed on the haematopoietic stem cells of the fetal liver at the 15th day of gestation[3], just before the onset of haematopoiesis and collagen production in the spleen[4]. The expression of this antigen on human leukaemic blast cells has, furthermore, been correlated with low levels of sialic acid on the surface of these tumour cells[5]. As fetal determinants can be expressed on adult bone marrow in disease states not involving malignant transformation[6], it is of interest to know whether production of this oncofetal antigen is repressed, or whether the determinants are in cryptic form at the plasma membrane due to some masking phenomenon. The investigation described here demonstrates that determinants which cross-react immunologically with fetal liver tissue can be enzymatically unmasked on normal adult murine haematopoietic stem-cell surfaces. This finding suggests that the expression of these oncofetal antigens on malignant cells does not depend on *de novo* production of the antigens, and that these determinants may play some part in normal differentiation control mechanisms.

We used adult bone marrow (ABM) cells from the femurs of 16-week-old male mice and 15-day fetal liver (FL) cells from fetuses of primigravida BALB/c and C57BL/6 mice. Following fractionation on a discontinuous albumin gradient to isolate the haematopoietic stem-cell fractions[7], ABM or FL stem cells were incubated individually or in sequence with collagenase (Calbiochem; grade A) and neuraminidase (Behring, from *Vibrio cholerae*) at 37°C for 30 min per enzyme. Cells were washed three times after each incubation. Collagenase incubation and washes were carried-out with 0.05 M Tris-HCl buffer, *p*H 7.6, containing 0.005 M $CaCl_2$. Neuraminidase buffer, *p*H 7.2, contained 0.004 M Na acetate, 0.116 M NaCl and 0.005 M $CaCl_2$. Enzyme incubations were carried out with constant mixing of cell suspensions on a Junior Orbit bath shaker at 200 r.p.m.

Subsequently, the enzyme-treated target (ABM and FL) cells were incubated with normal rabbit serum or with a rabbit antiserum which was raised against BALB/c FL cells (10^7 cells, subcutaneously, weekly for 10 weeks) denoted as rabbit anti-mouse fetal liver serum. After appropriate absorptions, this latter antiserum did not react with adult murine spleen, liver or ABM cells. Indirect immunofluorescence was then carried out using a previously described method[5]. Cells for the electron microprobe (X-ray fluorescence) analysis were reacted with ferritin-tagged antibody or normal globulin. Single cells were analysed in a modified Hitachi HU 10A transmission electron microscope which was adapted with a high resolution lithium-drifted silicon (Si(Li)) detector. Computer analysis of the intensity of the $K\alpha$ peak for iron (6.4 KeV) determined the number of ferritin-tagged antibody molecules bound by cell membrane determinants[8,9]. The quantitative expression of fetal antigen determinants was correlated to the respective enzyme treatment(s).

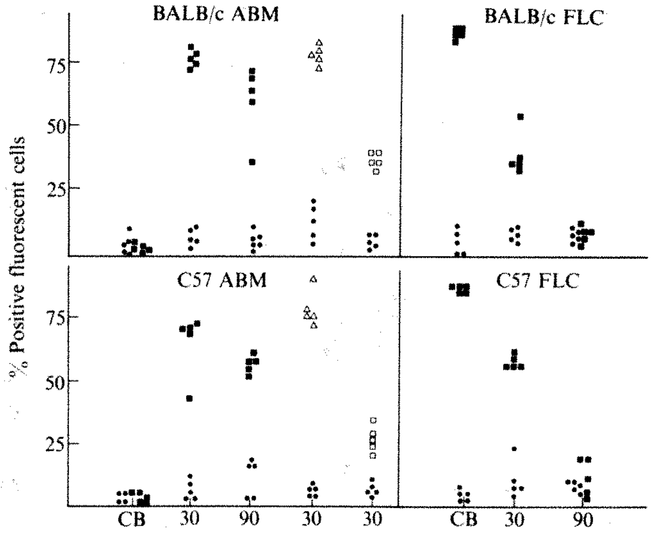

Fig. 1 Enzymatic modulation of cell-surface expression of fetal antigen determinants assayed by indirect immunofluorescent labelling. Mouse ABM or FL cells were treated with control buffer (CB) or with 30 or 90 U collagenase, followed by 12.5 U neuraminidase, and then incubated with normal rabbit serum (●), rabbit anti-mouse fetal liver serum (■), antiserum absorbed with adult mouse tissue (△) or antiserum absorbed with mouse fetal liver (□). Each point represents one experiment evaluating 100 cells.

Figure 1 demonstrates the effect of sequential incubations of ABM cells with collagenase and neuraminidase. The optimal regimen for uncovering fetal determinants on BALB/c ABM cells was 30.0 U collagenase followed by 12.5 U neuraminidase. After this enzyme treatment 80% of the ABM cells were labelled by rabbit anti-mouse fetal liver serum. There was no

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* To whom reprint requests should be sent.

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Table 1 Quantitation of membrane-associated fetal determinants

BALB/c target cell*	Enzyme treatment†	Serum*	No. of fetal determinants per cell (by X-ray)‡	% Positive fluorescent cells X-ray	% Positive fluorescent cells Indirect IF‡
FL	None	RAMFLS	$1.2 \pm 0.6 \times 10^6$	96	89 ± 2
FL	None	NRS	$1.0 \pm 0.05 \times 10^4$	8	4 ± 3
ABM	None	RAMFLS	$1.6 \pm 0.05 \times 10^4$	18	4 ± 3
ABM	None	NRS	$0.9 \pm 0.05 \times 10^4$	15	4 ± 4
ABM	N/C	RAMFLS	$1.0 \pm 0.2 \times 10^4$	18	19 ± 2
ABM	N/C	NRS	$1.3 \pm 0.2 \times 10^4$	12	8 ± 2
ABM	C/N	RAMFLS	$1.6 \pm 0.5 \times 10^5$	74	80 ± 4
ABM	C/N	NRS	$1.2 \pm 0.4 \times 10^4$	22	8 ± 3
ABM	C/N	RAMFLS (absorbed FL)	$0.8 \pm 0.03 \times 10^4$	28	39 ± 3

* ABM Adult bone marrow; FL, fetal liver; RAMFLS, rabbit anti-mouse fetal liver serum; NRS, normal rabbit serum.

† Target cells were incubated with 30 U collagenase (C) and 12.5 U neuraminidase (N), each for 30 min at 37°C, in the sequence indicated.

‡ Mean ± s.d.; $n = 50$ for X-ray fluorescence, assays done one cell at a time; $n = 100$ for immunofluorescence (IF), assays repeated five times.

increase in nonspecific labelling of cells by normal rabbit serum. C57BL/6 ABM cells reacted in the same manner, showing that detection of the fetal determinants was not restricted to $H\text{–}2^d$ allelic expression. The reverse enzyme sequence or treatment with either enzyme alone did not increase the reactivity of the ABM cells with the rabbit antiserum.

The rabbit anti-fetal serum was absorbed twice with FL cells (10^7 cells ml^{-1}, 1 h at 37°C, 16 h at 4°C), and then incubated with syngeneic (either BALB/c or C57BL/6) ABM cells which had been subjected to the optimal enzyme regimen. As shown in Fig. 1, the number of unmasked ABM cells labelled by the absorbed serum fell by 50% for BALB/c and by 67% for C57BL/6 cells. Specificity controls showed that absorption with other adult cells (spleen, liver, intestine or thymus) produced no reduction in labelling of unmasked ABM by the antiserum. Furthermore, these tissues were not labelled by the antiserum after similar enzyme treatment.

As demonstrated in Fig. 1, rabbit antiserum reacted optimally with fetal liver cells which were not enzymatically modified (90% fluorescent cells). The incubation of FL cells with collagenase followed by neuraminidase resulted in a decrease in the number of cells labelled by the antiserum as the concentration of collagenase was increased. In contrast to ABM cells, fetal antigen expression on FL cells could be modified by treatment with either enzyme alone.

X-ray immunoflurescence spectrometry has allowed measurement of the rabbit antiserum-reactive determinants on the cell surface (Table 1). Fetal determinants expressed on untreated FL cells averaged 10^6 determinants per cell. ABM cells at the optimal conditions for unmasking displayed approximately an order of magnitude fewer determinants than the FL cells. Untreated ABM cell controls or ABM cells treated with neuraminidase followed by collagenase displayed approximately 10^4 determinants per cell (2 orders of magnitude below untreated FL cells). Table 1 also presents data comparing the number of cells which displayed significant antibody binding (>95% confidence) as determined by X-ray fluorescence with the number of positive cells in the indirect immunofluorescence assay. Although the reactivity of anti-fetal serum for unmasked ABM was not completely abolished in the indirect fluorescence assay after absorption with FL cells, X-ray analysis revealed that the percentage of positive cells and the number of fetal determinants per cell labelled by the absorbed antibody were comparable to background levels with normal rabbit serum.

The mechanism(s) responsible for the unmasking of fetal determinants on the surface of the ABM cell, or for their removal from the FL cell surface, has not been determined. In our system for unmasking, collagenase treatment must precede neuraminidase. After collagenase has acted on its substrate(s), neuraminidase can cleave sialic acid. Whether the cleavage of sialic acid occurs from unmasked glycoprotein, glycolipid or some other macromolecules was not determined. The unmasking may be caused by simple removal of components from the cell surface or could also involve conformational changes at the plasma membrane. An enzymatic function is presumed necessary because heat-inactivated enzymes were ineffective. Other proteolytic enzymes (papain and trypsin) were tested (alone or in combination with neuraminidase) and failed to achieve similar unmasking.

The phenotypic expression of fetal haematopoietic stem-cell determinants on the ABM cell surface after enzymatic unmasking suggests that their transcription is not repressed by the ABM cell. The unmasking of fetal determinants on ABM cells by collagenase and neuraminidase was unique among the tissues studied (adult intestine, liver, spleen and thymus) and enzymes tested. However, it is possible that the expression of these or similar determinants may occur in other conditions, for example, as a function of differentiation or de-differentiation–skin[10,11], muscle and lung fibroblasts[12] have all been shown to express fetal determinants when cultured *in vitro*.

Mid-gestation fetal liver cells cross-react immunologically with a variety of viral and chemically induced animal tumours[13–15], as well as with human malignant melanoma[16], leukaemia[5,16] and colon carcinoma[17]. As transformed cells of various histological types express fetal determinants, it may be physiologically significant that only adult bone marrow cells were demonstrated to carry these determinants in a cryptic fashion.

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A virus-free immunogen effective against Epstein-Barr virus

David A. Thorley-Lawson

Sidney Farber Cancer Institute, Harvard Medical School, 44 Binney Street, Boston, Massachusetts 02115

Epstein–Barr virus (EBV)-associated diseases—infectious mononucleosis (IM), nasopharyngeal carcinoma (NPC) and Burkitt's lymphoma (BL)—constitute considerable world health problems. EBV infection occurs in all human populations and is accompanied by seroconversion and life-long persistence of the virus[1]. The antibodies present as a consequence of infection are considered important in controlling the persistent viral infection and in preventing recurrent bouts of IM which is known to be caused by the virus[2]. As EBV infection during childhood does not usually result in illness[20], only seronegative adolescents and young adults are susceptible to IM. Similarly, the groups at risk to EBV-associated neoplasms are known. The first group includes children in areas of hyper- and holoendemic malaria in Africa[3], where BL is the most common childhood cancer. The other group consists of people, particularly men, of the HLA-2 and Sin 2 haplotypes[4], of southern Chinese origin amongst whom NPC is the most common tumour[5]. It has been suggested that an anti-EBV vaccine should be developed[6], and this debate has been stimulated recently by a study further implicating EBV with African BL[7]. I report here the successful production of a DNA-free immunogen which may be readily prepared in large quantities and will successfully invoke a strong neutralising anti-EBV response in experimental animals. This material could form the basis for developing an anti-EBV vaccination programme.

A vaccine against EBV has been considered for several reasons. First, vaccines made from viral antigens have proved highly successful in protecting against tumours caused by Marek's disease herpesvirus in chickens[8] and by herpesvirus saimiri in marmosets[9]. Second, as stated above, EBV-associated diseases constitute considerable world health problems[2,3,5] and the populations at risk have been defined. Thirdly, the significance of EBV as a tumorigenic agent in humans could be finally established if it were possible to prevent tumours by vaccination.

Work in this laboratory has been concerned with studying the biochemical nature of the EBV-associated membrane–neutralising antigen complex and using this information to develop an effective anti-EBV vaccine. It was shown previously that a rabbit anti-EBV serum prepared by immunisation with whole purified virus possessed a high neutralising antibody titre[10]. It was not possible to use purified virus as a source for the biochemical characterisation of the antigens recognised by these antibodies because of the technical difficulties involved in concentrating and purifying the small amount of virus released

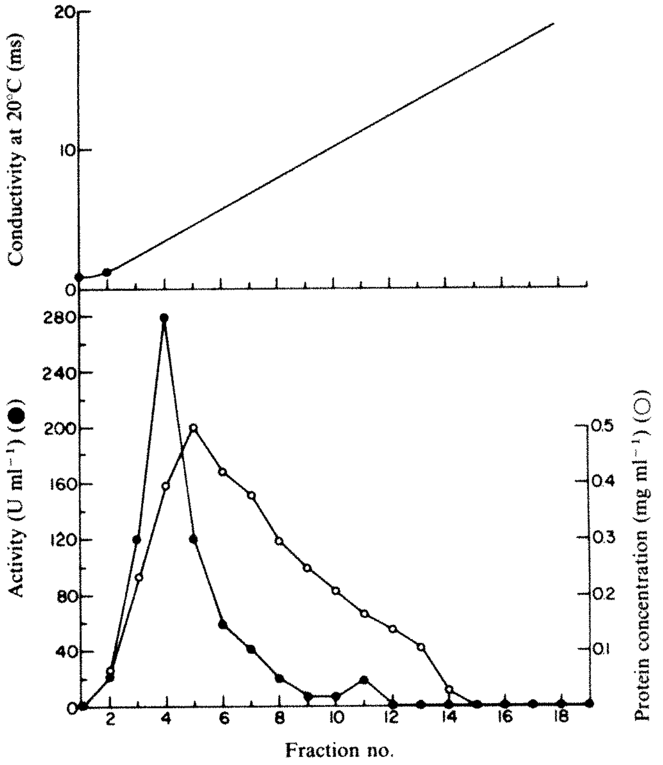

Fig. 1 Fractionation of EBV membrane antigens by DE-52 ion-exchange chromatography. Plasma membranes were prepared essentially as described previously[18] (full details to be published elsewhere). The plasma membranes (20–30 mg of protein from A_{260}/A_{280}) from ~15 g of P3HR-1 cells grown in restrictive conditions were resuspended in 10 mM Tris *p*H 7.4, 0.25 mM dithiothreitol (DTT) and solubilised by the addition of Triton X-100 (3:1 w/w, Triton X-100:protein) for 30 min at 4 °C. The solution was clarified by centrifugation at 200,000 *g* for 1 h and the supernatant applied to a 4 ml column of DE-52 previously equilibrated with 10 mM Tris *p*H 7.4, 0.25 mM DTT and 0.2% Triton X-100. The column was washed with 2–3 column volumes and the bound material eluted with a linear gradient (1 ml fractions, 15 ml h^{-1}) of 0–0.4 M potassium phosphate *p*H 7.4 in the equilibrium buffer. Fractions were all dialysed against phosphate buffered saline (PBS) before assay. Protein (○) was estimated by the method of Lowry[14] and EBV membrane antigens (●) by competition of the ^{125}I-PrA assay (see Fig. 2). Approximately 5.52 mg of protein and 2,600 units of membrane antigen were applied to the column.

Table 1 Effect of adding the neutralising (anti-P3HR-1) and control (anti-RAMOS) antisera at various times pre- and post-infection

		Infectivity assayed by:			
		DNA synthesis† (^{3}H-thymidine incorporation)		Outgrowth‡ (no. of wells with transformed foci)	
Time antibody added*	Addition of virus	Anti-P3HR-1	Anti-RAMOS	Anti-P3HR-1	Anti-RAMOS
1 h pre-infection	+	160	4,900	0/6	6/6
2 h post-infection	+	8,100	9,300	6/6	6/6
24 h post-infection	+	7,900	7,200	6/6	6/6
No antibody added	+	7,400		6/6	
No antibody added	−	100		0/6	

*Antisera were used at a dilution of 1:15.
†For experimental procedure see Fig. 3 legend. Each value was the mean of triplicate experiment, error ≤10%.
‡For experimental procedure see Fig. 4 legend.

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by producer cell lines. However, the neutralising antibodies could be specifically absorbed by EBV producer cell lines[10]. Therefore, the neutralising antigens are expressed on the plasma membranes of these permanently growing lymphoblastoid cell lines which may be cultured to indefinitely large volumes, thus providing a potential source of large amounts of the antigens. Furthermore, the antigen could be assayed for quantitatively by means of the ^{125}I-labelled *Staphylococcus aureus* protein (^{125}I-PrA) assay[10] and it has been possible to purify and identify components of the membrane antigen using this assay. The antigen comprises a glycoprotein complex of at least three major (molecular weights (MW) 350,000, 140,000 and 75,000) and several minor polypeptides present on the plasma membrane of all producer cell lines[11] and on the virion itself (unpublished results). It was decided, therefore, to try to immunise rabbits specifically against EBV using a partially purified preparation which contained this complex of polypeptides.

Plasma membranes were purified from the P3HR-1 cell line by nitrogen cavitation followed by differential sucrose density centrifugation. The resulting purified plasma membranes contained 4–6% DNA by weight as judged by A_{260}/A_{280} (ref. 12). They were solubilised with the non-ionic detergent Triton X-100 and passed over a DE-52 ion exchange column to remove the residual DNA[13]. The material which bound to the column was eluted with a salt gradient. The fractions were assayed for protein by the method of Lowry as modified by Dulley and Grieve[14] and for EBV-specific membrane antigens by inhibition of the ^{125}I-PrA binding assay[11] (Fig. 1). The tubes with peak activity were pooled, assayed for DNA content[15] and found to have 0.25% DNA by weight compared to Lowry protein (salmon sperm DNA and bovine serum albumin were used as standards).

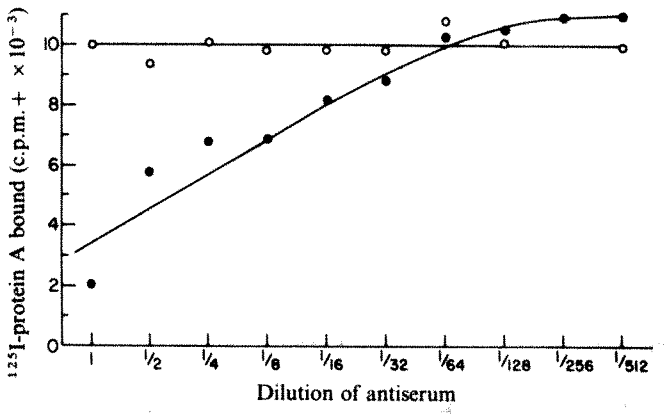

Fig. 2 Assay for the purified (from P3HR-1) and mock-purified (from RAMOS) EBV membrane antigen using competition of the ^{125}I-labelled staphylococcal protein A assay. The appropriately diluted antigen (50 μl) in a final solution of 0.1% Triton X-100 in PBS was incubated overnight with antibody (10 μl of 1:8 diluted rabbit anti-EBV serum) at 4 °C. The antibody activity in duplicate 25 μl aliquots was then assayed as described previously[10] except that the P3HR-1 cells used in the assay were first fixed with glutaraldehyde. The activity in units may be calculated as follows: the dilution of antigen required to inhibit the assay by

$$\frac{\text{total volume of sample } (\mu\text{l})}{50}.$$

In this experiment an antigen dilution of 1 is equivalent to 160 μg per ml of membrane protein. A background of 2,600 c.p.m. obtained with normal rabbit serum has been subtracted from these data. ●, Antigens purified from producer cell line P3HR-1; ○, antigens mock-purified from EBV-negative cell line RAMOS.

A mock purification was performed using the EBV-negative lymphoblastoid cell line RAMOS. The pooled fractions from P3HR-1 and RAMOS were then tested for their ability to compete for the rabbit anti-EBV serum in the ^{125}I-PrA binding assay (Fig. 2). Only the preparation from P3HR-1 was able to compete, indicating that this material contained the specific EBV membrane antigens.

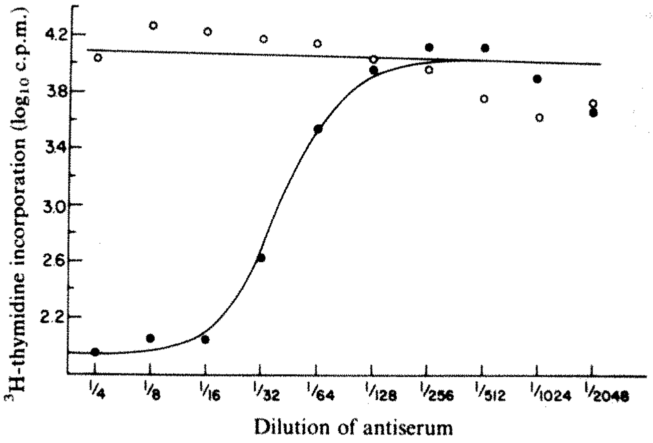

Fig. 3 Neutralisation of EBV infectivity by serum from immunised and mock-immunised rabbits. Infectivity was assayed by measuring the stimulation of DNA synthesis in resting peripheral B lymphocytes. Rabbits were immunised with partially purified antigens from a DE-52 column (Figs 1, 2). The material, consisting of ~100 μg of protein in 0.5 ml of 0.1% Triton X-100 and PBS, was emulsified in complete Freund's adjuvant and inoculated subcutaneously in the neck and intramuscularly in the thigh. The procedure was repeated three times over the course of two months and the animals were bled 10 days after the last injection. Before use in the neutralisation assay sera were absorbed 4 times for 1 h at room temperature with an equal volume of fresh peripheral lymphocytes to remove nonspecific cytotoxic activity from the sera. The methodology for assaying neutralising activity in sera has been described in detail elsewhere[10]. Briefly, the B95–8 strain of virus and sera were incubated for 1 h at 37 °C and then added to fresh B lymphocytes purified from the blood of normal adult humans by immunoabsorbent chromatography. The cells were then incubated for 1 week and proliferation assayed by measuring incorporation of ^{3}H-thymidine. Each experiment was performed in triplicate. ●, Antiserum prepared from a rabbit immunised with partially purified EBV membrane antigens from the EBV producer cell line P3HR-1; ○, antiserum prepared from a rabbit immunised with mock-purified EBV membrane antigens from the EBV-negative cell line RAMOS.

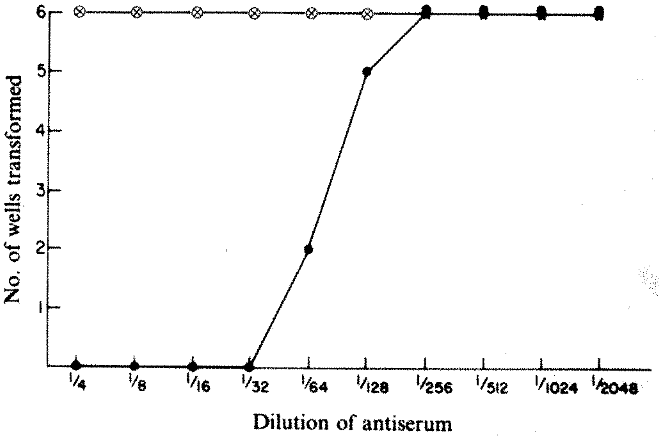

Fig. 4 Neutralisation of EBV infectivity by serum from immunised and mock-immunised rabbits. Infectivity was assayed by measuring the outgrowth of transformed foci. Details as for Fig. 3 legend, except infectivity was assayed by setting up 6 culture wells for each point and counting the number of wells containing transformed foci of proliferating lymphoblasts 20 days postinfection[10]. ●, Antiserum prepared from a rabbit immunised with partially purified EBV membrane antigens from the EBV-producer cell line P3HR-1. ○, Antiserum prepared from a rabbit immunised with mock-purified EBV membrane antigen from the EBV-negative cell line RAMOS. ×, Normal rabbit serum.

Rabbits were immunised with the partially purified material from RAMOS and P3HR-1 and the resulting antisera were tested for their ability to neutralise the virus *in vitro*. EBV from the B95–8 lymphoblastoid cell line was preincubated with the different sera at various dilutions and the mixture was then used to infect peripheral B lymphocytes from normal adult donors. The infectivity was estimated by measuring either the stimulation of cellular DNA synthesis in the B cells (Fig. 3) or the outgrowth of foci of transformed lymphoblasts[16] (Fig. 4). In both experiments the virus infectivity was neutralised by the antiserum (titre 1:50 to 1:100) raised by immunising with the partially purified EBV membrane antigens from P3HR-1. The equivalent serum raised against mock-purified antigens from the RAMOS cell line was completely ineffective, as was preimmune normal rabbit serum. When the antiserum against P3HR-1 was added a few hours postinfection (Table 1), it was completely ineffective in preventing the infection. These results suggest that the antiserum was specifically neutralising the virus and was not toxic for the infected cells.

These results demonstrate that it is possible to generate a specific EBV-neutralising antibody response in experimental animals with a DNA-free preparation of partially purified membrane antigens from the plasma membranes of EBV-producer cell lines. These results are in agreement with previous observations[19] that EBV membrane antigens removed from super-infected RAJI by papain digestion would invoke an EBV-neutralising response. The production of EBV membrane antigens by super-infection will, however, be difficult to scale up. By comparison, the source of membrane antigens in the present study was EBV producer lymphoblastoid cell lines. As these cell lines are permanently established in culture, they provide a potentially limitless source of the EBV membrane antigens. In addition, the culture conditions and purification procedures used were simple and will be amenable to scaling up for production of large amounts of vaccine.

As well as the above mentioned associations with neoplasia in man, EBV has been shown to cause tumours in certain New World monkeys, notably marmosets[17]. This provides further support for the suggested etiological role of EBV in human carcinogenesis. The next test for the immunogen described here will be to assess its ability to immunise these monkeys against EBV-induced neoplasia.

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Immunoregulatory effects of a covalent antigen–antibody complex

R. B. Taylor & J. P. Tite

MRC Immunobiology Group, Department of Pathology, The Medical School, University Walk, Bristol, UK

C. Manzo

Division de Immunologia, Fondazione Senatore Pascale, Istituto per lo Studio e la Cura dei Tumori, Capella dei Cangiani, Napoli, Italy

It is becoming increasingly clear that any specific immune response must be subject to a control mechanism of the same order of specificity. The basis of such specificity in control almost certainly lies in the antibodies themselves, whether free or cell bound. The response to a given antigen might then be regulated by antibodies specific either for the antigen ('Ab 1') or for the variable regions (idiotypes) of Ab 1. Whereas the latter can interact directly with the binding sites of cellular receptors for antigen, the former can only do so if the antigen is also present to act as a bridge. Furthermore, because an anti-idiotype antiserum is usually prepared against a monoclonal antibody, it will only be able to influence directly those clones of cells which carry the chosen idiotype, and thus may leave unaffected other clones which are specific for the same antigen but which carry different idiotypes. An immune complex, on the other hand, should influence all clones sensitive to the antigen in question. Experimentally, the injection of antigen–antibody complexes can either increase[1,2] or decrease[3,4] the subsequent response to antigen. These effects often require the presence of an intact Fc region[4] and may differ according to the class (or isotype) of the antibody[5]. The injection of anti-idiotype antisera can have striking effects on the expression of particular idiotypes, but usually has little effect on the overall response. Again, these effects require the participation of the Fc region of the anti-idiotype antibody[6], and can vary according to its isotype[7,8]. To study the regulatory effects of antibody more closely, we have attempted to prepare immune complexes which could influence the overall response, and yet have effects as powerful as those obtained with anti-idiotype antibodies. The immune complexes obtained were of the defined constitution Ag_2Ab, in which stability was ensured by linking antigen to antibody through a covalent bond. We report here that, depending on the conditions used, these complexes induced either priming or tolerance of the response of mice towards subsequent injections of antigen alone. We consider that such complexes could be useful not only as tool for studying immunoregulation, but also as a practical means of influencing immune responses.

The complexes were prepared by mixing hapten–conjugates of the chosen antigen with anti-hapten antibody. Cross-linking was minimised by the use of conjugates which were as far as possible monosubstituted with the hapten. The complexes were rendered stable by the introduction of a covalent bond through the use of the photosensitive affinity label 'NAP' (4-azido-2-nitrophenyl)[9] as the hapten. As the antigen now becomes effectively an extension of the antibody variable region (Fig. 1), such a complex should mimic an anti-idiotype antibody in its mode of interaction with cellular receptors. The method is summarised in Fig. 1 legend and will be reported in detail elsewhere. As antigen we used pancreatic ribonuclease D (RNase; Sigma).

In the first experiment young adult mice of the hybrid strain (BALB/c × CBA/H)F_1 were each injected intravenously with a single dose of either complex containing 0.16–100 μg of complexed anti-NAP, or free RNase equal to that contained in 4 μg or 100 μg complex, or were left uninjected. Six weeks later the mice were each challenged intraperitoneally with 50 μg of RNase in complete Freund's adjuvant. They were bled 2 weeks

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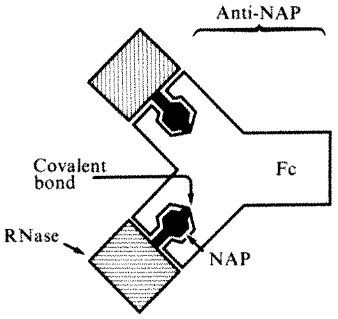

Fig. 1 Diagram of a molecule of covalent complex of NAP-RNase with anti-NAP. NAP was first converted into a form which on the one hand was more reactive with proteins in physiological conditions, and on the other included a spacer chain so as to increase the likelihood of its being sufficiently exposed to interact with the antibody. This was done by reacting NAP with aminocaproic acid in the presence of sodium carbonate, as described by Fleet *et al.*[9] for NAP-lysine. The resulting NAP aminocaproic acid (NAP-cap) was coupled to *N*-hydroxysuccinimide to form the succinimide ester (NAPcapOSu) using dicyclohexylcarbodiimide, as described by Becker and Mäkelä[13] for the analogous DNPcapOSu. The concentration was estimated by optical density after dilution in 0.05 M glycine, *p*H 8.5, assuming E_{M}^{485} = 4,800. An aliquot of NAPcapOSu was stirred rapidly with a 2 molar equivalent of RNase at 10 mg ml^{-1} in 0.05 M HEPES, *p*H 7.5, for 1–2 h at room temperature. Uncombined hapten was removed by passage through a Sephadex G-25 column. As RNase has one lysine side chain which is much more reactive than the others at *p*H values close to neutral[14], it was thought likely that this treatment would result in very few molecules having more than one NAP attached. Anti-NAP antibody was prepared in rabbits by injection of NAPcap–keyhole limpet haemocyanin (9 haptens per 100,000 molecular weight) in complete Freund's adjuvant. It was purified by elution with hapten from an immunoadsorbent column. NAPcap–RNase, prepared with RNase trace labelled with ^{125}I, was mixed with anti-NAP antibody in a 2 molar excess of NAP to the antibody-binding sites, and the mixture was illuminated with a standard 60-W bulb for 15 min. The antibody was then concentrated by precipitation with 50% saturated ammonium sulphate, redissolved in a small volume and passed through a gel-permeation column of AcA 34 (LKB). The radioactivity served to monitor RNase, and optical density at 280 nm was mainly due to anti-NAP. It was found that some of the RNase eluted with the anti-NAP whereas the rest followed in the position expected for free RNase. The concentration of RNase in the pooled first peak was obtained from the radioactivity, and multiplied by the ratio of molecular weights to give the concentration of complex, expressed in terms of complexed anti-NAP.

later and their sera titrated for anti-RNase antibody activity by a modification of Farr's ammonium sulphate procedure[10], using ^{125}I-labelled RNase at 10^{-8}M. The results (Fig. 2) show that whereas RNase alone had little or no priming effect, the activity of the complex was extremely strong. It was detectable at the 0.16 μg dose of complex (containing 0.027 μg RNase) and rose to a maximum at the 20 μg dose. For subsequent experiments 10 μg was chosen as the minimal dose required for optimal effect.

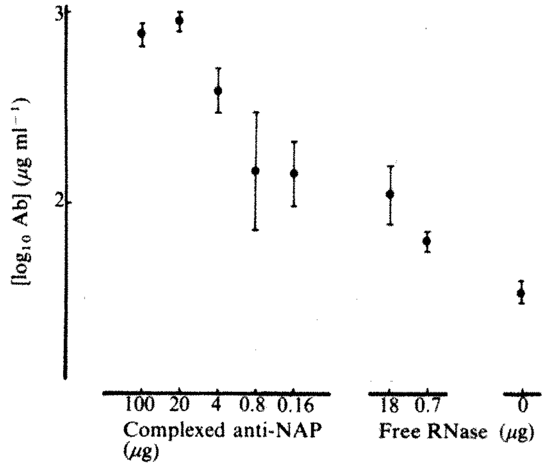

Fig. 2 Dose curve of priming by complex. (BALB/c×CBA/H)F$_1$ mice were injected intravenously with the doses of RNase complex or free RNase as indicated, 6 weeks before challenge with 50 μg RNase in complete Freund's adjuvant. Sera from a day 14 bleed were assayed by the Farr technique. Each group contains six mice.

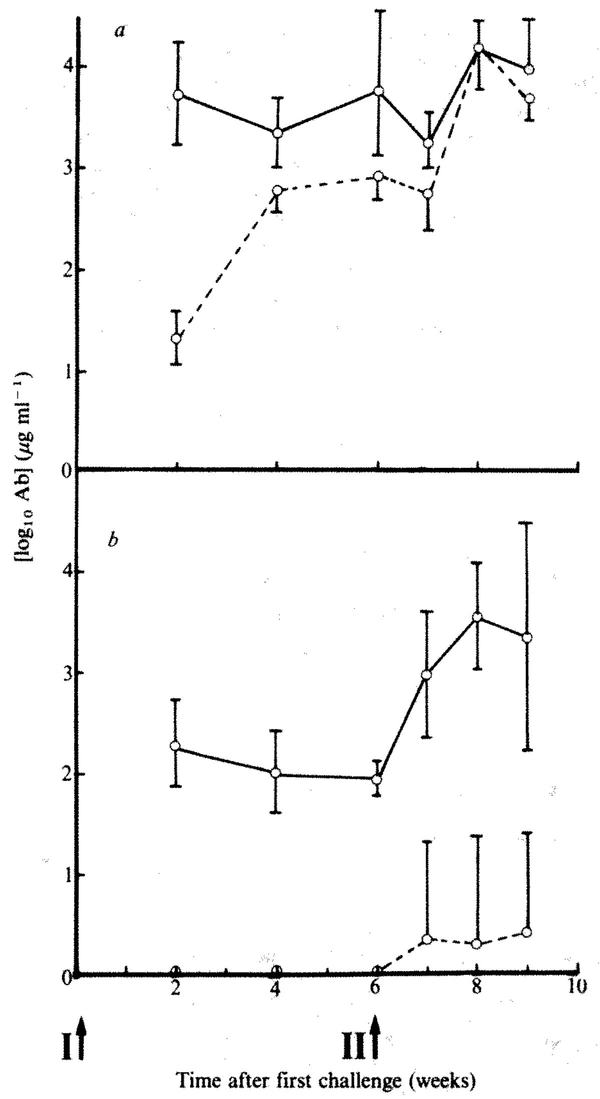

Fig. 3 Challenge with or without adjuvant. Mice were either primed with 10 μg RNase complex (O—O) or left unprimed (O- - - -O). Six weeks later they were challenged with 100 μg RNase (I↑) either in complete Freund's adjuvant (*a*) or in saline (*b*). After a further 6 weeks they received a second challenge (II↑) of 100 μg RNase in incomplete Freund's adjuvant (*a*) or saline (*b*).

The second experiment shows that adjuvant is not necessary to recall the priming induced with covalent immune complex, as this can also be done with soluble antigen. Complex-primed or control mice were challenged with 100 μg RNase in either complete Freund's adjuvant (Fig. 3*a*) or saline (Fig. 3*b*). Although unprimed mice made no detectable antibody in response to the first challenge with soluble RNase, complex-primed mice made over 100 μg ml^{-1}. After a second challenge a few of the controls showed a small response, but the primed mice made about 5 mg ml^{-1} antibody, (over 1,000 times more than controls) and reached about the same level as the maximum achieved by the adjuvant-challenged groups. With adjuvant challenge (Fig. 3*a*) this maximum titre was achieved by the complex-primed group as early as 14 d after the first challenge, and was not increased further by a second challenge. By contrast, the titres of unprimed mice rose much more slowly, although after the second challenge they did eventually match those of the complex-primed mice.

It seemed possible that this remarkable priming effect was dependent on the rabbit antibody being itself a foreign protein. To test this, some mice were made tolerant to rabbit IgG by intraperitoneal injection of 1 mg of an ultracentrifuged preparation of the fraction precipitating between 0.81 and 0.92 M sodium sulphate[11]. One week later they were injected with 10 μg RNase complex as before. Controls received complex alone, equivalent RNase, or no injection. After challenge 6 weeks later with RNase in complete Freund's adjuvant (Fig. 4), the priming effect was again seen in the mice receiving complex alone. By contrast, the mice which had previously been made tolerant to rabbit IgG showed a marked suppression of the response to RNase. Both the priming and suppressive effects were still present, although somewhat diminished, 10 weeks after the first challenge, but were almost abolished after a second challenge.

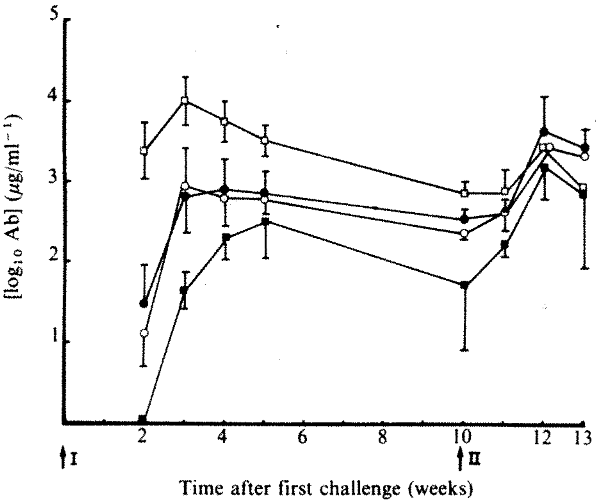

Fig. 4 Effect of prior tolerance to rabbit IgG. One group of mice was injected with 1 mg of rabbit IgG, prepared as described in the text (■), and the other three groups were left uninjected. One week later the four groups received 10 μg RNase complex (□, ■), 2 μg RNase (●), or no injection (○). All groups were challenged twice, at 6 and 16 weeks later, with 100 μg RNase. The first challenge was with complete (I↑) and the second with incomplete (II↑) Freund's adjuvant.

The results described here demonstrate that powerful immunoregulatory effects can be obtained with a covalent antigen–antibody complex. Because rabbit antibody was used in these experiments, it was first expected that the complexes would suppress the response to RNase, by analogy with the suppressive effect of injecting rabbit antibody to a mouse idiotype[12]. However, there are several differences between these two situations which may be significant. One of these is the fact that, whereas the complex was prepared from purified antibody, the anti-idiotype was administered as a whole immunoglobulin fraction, and thus would have contained nonspecific immunoglobulin in considerable excess to the anti-idiotype antibody. This might have had the same effect as the prior injection of rabbit IgG in our experiment. However, our unpublished experiments using anti-NAPs prepared in various species indicate that the recognition of foreign epitopes on the immunoglobulin is by no means the only factor which determines whether the outcome of injecting such covalent complexes is one of priming or suppression. For example, priming effects have been obtained with complexes formed from an IgG1 fraction of isologous mouse anti-NAP.

We have also studied the mechanism of action of the rabbit anti-NAP–NAP–RNase complex, and found that the Fc portion of the molecule is essential for its immunoregulatory effects, and that covalent bonding greatly enhances these effects, but is not essential for them; these studies will be reported elsewhere. We have obtained equally strong priming effects with several antigens other than RNase, suggesting that this may possibly represent a practical immunisation schedule which could have particular value in that it completely avoids the use of adjuvants.

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Graft-versus-leukaemia reactivity induced by alloimmunisation without augmentation of graft-versus-host reactivity

Mortimer M. Bortin & Robert L. Truitt*
May & Sigmund Winter Research Laboratory, Mount Sinai Medical Center, Milwaukee, Wisconsin 53226

Alfred A. Rimm
Department of Biostatistics/Epidemiology, Medical College of Wisconsin, Milwaukee, Wisconsin 53226

Fritz H. Bach
Immunobiology Research Center, University of Wisconsin, Madison, Wisconsin 53706

Supralethal doses of chemoradiotherapy followed by allogeneic bone marrow transplantation are being used to treat patients with acute leukaemia refractory to conventional chemotherapy regimens[1-5]. Despite this approach, leukaemia has recurred in about one-third of the patients[1-5]. One strategy to solve this problem would be to transplant marrow with antileukaemic reactivity to obtain a graft-versus-leukaemia (GvL) effect against residual leukaemia cells[6]. Many workers have demonstrated antileukaemic effects following transplantation of immunocompetent cells from allogeneic donors in animal models, but graft-versus-host (GvH) disease proved to be a complication[6-8]. Recently, Weiden *et al.*[9] presented evidence of a GvL effect in human recipients of marrow grafts from HLA compatible allogeneic donors. Unfortunately, this antileukaemic effect was associated with moderate, severe or lethal GvH disease, and the lower probability of recurrent leukaemia was offset by a higher probability of GvH-related mortality[9]. We report here the results that suggest a clinically feasible method of obtaining desired GvL effects while avoiding undesired GvH reactions. Alloimmunisation of CBA (H-2^{k}) mice was found to induce a population of cells which, when transplanted into leukaemic AKR (H-2^{k}) mice, led to the destruction of disseminated leukaemia (AKR-L) cells. Furthermore, the transplanted cells caused no augmentation in the mild GvH disease observed when cells from unimmunised CBA donors were administered to lethally irradiated non-leukaemic AKR hosts.

Immunocompetent cells from CBA mice were tested for GvL reactivity in a bioassay system as follows: On day zero, 1×10^5 viable AKR-L cells (obtained by pooling fresh spleen cells from 15 AKR mice bearing spontaneous T cell acute lymphoblastic leukaemia) were inoculated intravenously (i.v.) into lethally

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irradiated (800 rad, total body 4h previous) 8–12-week-old AKR primary hosts. One day later, as the sole antileukaemic treatment, immunocompetent cells (2×10^7 spleen or 10^7 bone marrow plus 10^7 lymph node) from unimmunised, isoimmunised, specifically immunised or alloimmunised CBA donors were transplanted into the AKR primary hosts. The GvL reaction was allowed to proceed for 6 d. To determine whether any viable leukaemia cells remained all spleen cells from each AKR primary host were transferred intraperitoneally (i.p.) to an individual 8–9-week-old AKR secondary recipient on day 7. Using this protocol all but one of 427 control animals, that is, those not given cells from immunised donors, died of leukaemia by day 53. Thus, the proportion of mice alive on day 90 provided a rigorous test of the GvL reactivity of immunised CBA cells.

To evaluate GvH reactivity, 8–12-week-old non-leukaemic AKR mice were exposed to 800 rad total body irradiation and given i.v. injections of immunocompetent cells from unimmunised or immunised CBA donors. The AKR hosts were observed daily for clinical evidence of GvH disease; all deaths were attributed to GvH disease.

The results are shown in Table 1. No antileukaemic effect was observed if AKR primary hosts were given no treatment (group 1) or cells from unimmunised or isoimmunised CBA donors (groups 2 and 3). Cells from specifically immunised CBA donors (group 4) had significantly greater GvL reactivity ($P < 0.001$) than did cells from isoimmunised CBA mice (group 3). Significant GvL reactivity ($P < 0.01$) was induced in CBA donors following immunisation with DBA/1 cells (group 5) in comparison with unprimed CBA cells (group 2); although the difference in GvL reactivity was not significant in comparison with group 3, this may have been due to the small group sizes. Immunisation of CBA donors with lymphoid cells from the other six H–2 incompatible strains of mice (groups 6–11) in each instance induced significant GvL reactivity ($P < 0.001$) in comparison with group 3, as did either six or two immunisations of CBA donors with pooled lymphoid cells from the seven H–2 incompatible strains (groups 12, 13).

Table 1 Survival data from assays in which immunocompetent cells from CBA (H–2^k) donor mice were tested for GvL reactivity in leukaemic AKR (H–2^k) mice and for GvH reactivity in nonleukaemic AKR mice

Group	Donor of transplanted cells*	GvL reactivity of donor cells† No. alive at 90 d/ no. AKR mice transplanted (%)	GvH reactivity of donor cells No. alive at 90 d/ no. AKR mice transplanted (%)
1	None	1/308 (0)	3/155 (2)
2	CBA	0/108 (0)	52/77 (68)
3	CBA α CBA	0/11 (0)	11/12 (92)
4	CBA α AKR-L$_x$	36/39 (92)	2/11 (18)
5	CBA α DBA/1 (H–2^q)	2/12 (17)	12/12 (100)
6	CBA α SJL (H–2^s)	24/44 (55)	17/24 (71)
7	CBA α C57BL/10 (H–2^b)	17/23 (74)	12/12 (100)
8	CBA α B10.P (H–2^p)	10/12 (83)	11/12 (92)
9	CBA α B10.RIII (H–2^r)	10/12 (83)	12/12 (100)
10	CBA α BALB/c (H–2^d)	11/12 (92)	12/12 (100)
11	CBA α A.CA (H–2^f)	12/12 (100)	10/12 (83)
12	CBA α Pool × 6	12/12 (100)	10/12 (83)
13	CBA α Pool × 2	11/12 (92)	11/12 (92)

Irradiated (800 rad) leukaemic and nonleukaemic AKR hosts received 2×10^7 spleen or 10^7 bone marrow plus 10^7 lymph node cells i.v. from unimmunised (group 2), isoimmunised (group 3), specifically immunised (group 4) or alloimmunised (groups 5–13) CBA donors. χ^2 test was used for significant differences between groups.

* CBA donors received six weekly immunisations i.p. with 1×10^7 lymphoid cells from the indicated strains; the H–2 haplotype of the immunising strain is in parentheses. AKR leukaemia cells (group 4) were irradiated with 3,000 rad. For pool immunisation (groups 12 and 13), 1×10^7 lymphoid cells from each of the seven H–2-incompatible strains were combined and injected i.p.; mice in group 13 received only two immunisations. CBA mice were used as donors one week after the last immunisation.

† A bioassay system was used to measure the GvL reactivity of CBA donor cells. All deaths were due to leukaemia.

The theoretical and experimental bases for the GvL bioassay model and the rationale for the use of the spleen as the most sensitive bioassay organ have been described previously[10]. This bioassay allowed critical evaluation of GvL reactivity without the potentially confounding complications of radiation injury, failure to obtain engraftment and GvH disease. All of these complications, in addition to leukaemia, could have been the cause of death had the primary rather than the secondary recipient been evaluated for survival and autopsied at the time of death.

T lymphocytes appeared to be required for the GvL reaction. Rabbit anti-θ serum and complement treatment of spleen cells from CBA mice alloimmunised with SJL lymphoid cells abolished GvL reactivity (none of 11 mice survived 90 d), whereas treatment of these cells with normal rabbit serum and complement caused no diminution of GvL reactivity (7 of 10 mice survived 90 d). This observation does not exclude a role for natural killer (NK) cells, as it has been suggested that NK cells express low density thy-1 antigen[11] and that alloimmunisation augments NK activity[12].

Survival data from GvH assays are presented in Table 1. Cells from specifically immunised CBA mice (group 4) caused a significant increase in GvH-related mortality in comparison with cells from unimmunised (group 2) CBA donors ($P < 0.005$) or isoimmunised (group 3) CBA donors ($P < 0.001$). However, transplanted cells from alloimmunised CBA mice (groups 5–13) caused no significant increase in mortality in comparison with groups 2 or 3.

Bach *et al.*[13] first proposed pool-priming as a means of generating cytotoxic cells to tumour or virus-infected targets. Zarling *et al.*[14] subsequently demonstrated that lymphocytes sensitised *in vitro* to pooled allogeneic cells were cytotoxic to autologous human leukaemia cells *in vitro*. The findings reported here extend these observations by demonstrating that pool-alloimmunisation *in vivo* generated cells which, when transplanted, killed disseminated AKR-L cells *in vivo*.

Several possibilities exist to explain the effectiveness of alloimmunisation in the induction of GvL reactivity. Activation of T cells which help antigen-specific or nonspecific reactions against the leukaemia targets, activation of NK cells and/or sensitisation of cytotoxic T lymphocytes to alloantigens cross-reactive with leukaemia target antigens are all possibilities. Whatever the mechanism, immunisation of CBA mice with allogeneic H-2-incompatible cells induced GvL reactivity without augmenting the GvH response. To the extent that sensitisation to antigens on cells of a single allogeneic strain in some cases led to relatively weak GvL reactivity, pool-alloimmunisation may circumvent the need to understand which alloantigens are needed to induce the desired GvL effect.

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Complement-fixing IgG1 constitutes a new subclass of mouse IgG

Peter L. Ey*, Stephen J. Prowse & Charles R. Jenkin

Department of Microbiology and Immunology, The University of Adelaide, PO Box 498, Adelaide, 5001 South Australia

Four IgG isotypes (IgG1, IgG2a, IgG2b, IgG3) have been distinguished in the mouse[1-3]. The similarity of the physicochemical properties of the different isotypes has to a large extent prevented their purification from serum and this has complicated functional studies on IgG antibodies in sera from infected or immunised mice[1,2,4]. The availability of immunoglobulin-secreting plasmacytomas[5], however, has made the production of isotype-specific antisera possible; the use of these antisera has shown immunoglobulin of each isotype to exist in sera from normal animals[3,5,6]. IgG1 antibodies, partially separated from IgG2 by electrophoresis in agar, have been reported[2] to be incapable of sensitising sheep red blood cells (SRBC), passively coated with antigen, for lysis by complement (C) although the antibodies agglutinated the cells and also mediated passive cutaneous anaphylactic (PCA) reactions in the mouse. The IgG2 antibodies, which mediated PCA reactions in the guinea pig but not in the mouse, lysed the antigen-coated SRBC in the presence of mouse C. It seems on the basis of this single report that mouse IgG1 has been widely accepted[6-8] to be a 'non-C-fixing' class of immunoglobulin—one which, on association with antigen, is unable to activate C. We have now purified IgG1 from the serum of mice immunised with SRBC and in this report present evidence that the IgG1 antibodies are not only haemolytic in the presence of C, but that they apparently account for more than half of the SRBC-specific, C-fixing IgG antibodies in the serum of the immunised animals.

Six-week-old female LACA mice from a random-bred specific-pathogen-free colony were conventionalised for 3 weeks before being injected intravenously with 1×10^8 SRBC in 0.2 ml of saline on days 0 and 21. Blood was collected on day 32, pooled and the serum stored at −20 °C. IgG1, IgG2a and IgG2b immunoglobulin was purified from the serum by fractionation on protein A-Sepharose CL-4B (Pharmacia) as previously described[9]. The peak eluate fractions were pooled, dialysed against 0.15 M NaCl–15 mM NaN_3 and stored at 4 °C to minimise aggregation.

Each IgG preparation was titrated for SRBC-specific antibodies by haemagglutination as well as by C-dependent haemolysis. The haemolytic titrations (Fig. 1) showed that each preparation contained antibodies which were lytic for SRBC in the presence of guinea pig C. This activity was SRBC specific, since neither agglutination nor lysis was observed when human or guinea pig RBC were used as target cells and the activity could be removed by adsorption to SRBC but not to the other types of cells (data not shown). Because IgG1 antibodies were believed to be incapable of activating C (ref. 2), it was thought possible that the IgG1 preparation contained trace amounts of SRBC-specific IgM antibody or aggregated IgG1 which might bind to the cells and activate C nonspecifically, perhaps via the alternative pathway. Thus, all of the IgG1 immunoglobulin was chromatographed on Sephadex G-200 to separate IgM and aggregated IgG from unaggregated (monomeric) IgG molecules. As depicted in Fig. 2, this fractionation yielded a single, homogeneous peak of monomeric IgG1 which represented, within the limits of detection, all the protein applied to the column. All the detectable haemolytic and haemagglutinating antibody activity was associated with this monomeric IgG1 protein peak. The fractions representing the void volume, where IgM and aggregated IgG would be expected to elute, contained neither agglutinating nor haemolytic activity. The IgG1 haemolytic activity was therefore not due to 19S IgM antibody, to aggregated IgG1, or to monomeric (8S) IgM (an unlikely possibility) since the latter would have eluted with the leading edge of the IgG peak (the same column has been used successfully to resolve IgE and IgG1 antibodies, measured by PCA reactions[10]). Subsequent chromatography of samples of the IgG2a and (separately) the IgG2b indicated that these preparations similarly consisted of only monomeric IgG.

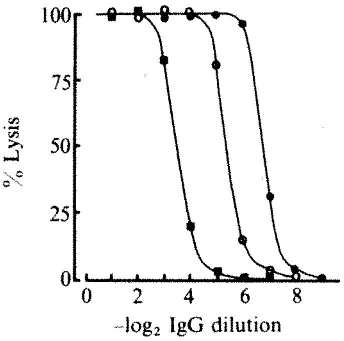

Fig. 1 Titration of IgG1, IgG2a and IgG2b for capacity to lyse SRBC in the presence of guinea pig C. All reagents were diluted in Veronal-buffered saline (*p*H 7.4) containing 0.15 mM $CaCl_2$ and 0.5 mM $MgCl_2$ (VB^{2+})[18], supplemented with 0.2 mg ml^{-1} bovine serum albumin and 7.5 mM NaN_3 (VB^{2+} − BSA). Fresh guinea pig serum, absorbed with SRBC (3 ml packed cells per 30 ml serum; 30 min, 0 °C). was stored at −20 °C in 0.25-ml aliquots which were thawed as required at room temperature and used immediately. Two-fold serial dilutions of antibody (0.2 ml) were mixed with 0.4 ml of 0.5% SRBC (3×10^7 cells) with rapid mixing. After incubation at 37 °C for 30 min, 0.2 ml of a 1 : 20 dilution of guinea pig serum was added. The tubes were incubated for 60 min at 37 °C, immediately centrifuged and the absorbance at 541 nm determined for each supernatant. Lysis is expressed relative to water-lysed (100%) and SRBC+C (0%) controls. No lysis of antibody-sensitised SRBC was observed in the absence of C. ●, IgG1; ○, IgG2a; ■, IgG2b.

Table 1 SRBC-specific antibody activity of IgG preparations

IgG*	Haemagglutination†	Haemolysis‡
IgG1	1/174	1/82.8 (1/146)
IgG2a	1/36	1/73.0 (1/93.6)
IgG2b	1/<1	1/12.8 (1/70.7)

* Analysis (μg per ml; protein, IgG1, IgG2a, IgG2b, IgM). IgG1: 568, 502, <1, <0.4, <0.08; IgG2a: 780, 5.7, 514, 1.24, 0.38; IgG2b: 181, 3.5, 8.08, 210, 0.79; Unfractionated serum: –, 2,580, 2,770, 930, 333. Protein concentrations estimated from A_{280} ($E_{1\%} = 14$), immunoglobulin levels by radioimmunoassay[9,16].

† Twofold serial dilutions of antibody (0.2 ml) in VB^{2+}–BSA (see Fig. 1 legend) mixed with SRBC (0.2 ml, 1%). Endpoint chosen as the maximum dilution causing agglutination, taken to a fraction (1/4, 1/2, 3/4, 1) ($\log_2$) of last positive well.

‡ Dilution of antibody causing 50% lysis using 1 : 20 C. Endpoints were determined by regression analysis of von Krogh plots[18]. Values in parentheses are the specific haemolytic titres calculated for a 1 mg ml^{-1} solution of each IgG.

To determine the purity of the IgG preparations, each was analysed by radioimmunoassay for immunoglobulin content. The IgG1 used for this analysis and for subsequent experiments was recovered from fractions 38–40 of the Sephadex G-200 fractionation (Fig. 2), corresponding to the top of the eluant IgG1 peak. The results of the analyses (Table 1 legend) showed that the IgG2a and IgG2b contained only minor amounts of other immunoglobulins totalling 0.94% and 6.8% by weight respectively. No contaminating immunoglobulin could be

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* To whom correspondence should be addressed.

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detected in the IgG1 preparation (IgM, IgG2a and IgG2b, together <0.26% by weight). The analyses corresponded closely to the protein concentrations, indicating little or no contamination by other serum proteins. This was confirmed by electrophoresis of each IgG (labelled with ^{125}I) on SDS-polyacrylamide gels[11] (not shown). In non-reducing conditions, the radioactivity migrated as a single band with a mobility characteristic of intact IgG. After reduction, each sample yielded only two peaks corresponding in mobility to heavy (γ) and light (L) chains. The L chain peak contained 24–28% of the total radioactivity (theoretically, 30%).

The SRBC-specific antibody titres of the IgG preparations are shown in Table 1. Most of the antibody, haemagglutinating and haemolytic, was associated with the IgG1. The specific haemolytic activities, shown in Table 1 in parentheses, indicated that on a weight basis the IgG1 possessed 1.56 and 2.06 times more haemolytic antibody than the IgG2a and IgG2b respectively. The lytic activity associated with the IgG1 could not therefore have been due to contaminating IgG2a or IgG2b, both of which were undetectable. From the concentrations of IgG1, IgG2a and IgG2b in the original serum pool (measured by radioimmunoassay; Table 1 legend) and the calculated specific haemolytic activity of each of the three IgG isotypes, it can be estimated that of the total SRBC-specific haemolytic IgG antibody present in the serum, 54% was due to IgG1, 37% to IgG2a and 9% to IgG2b. The validity of this calculation is conditional on the specific haemolytic activities of the IgG isotypes being unaltered by the purification procedure. The possibility of partial denaturation in the conditions used for elution from the protein A-Sepharose (IgG1, *p*H 5.8; IgG2a, *p*H 4.5; IgG2b, *p*H 3.5) cannot be excluded, although the fractionation was carried out at 4 °C and the fractions were neutralised as they were collected. The observed haemolytic activity of the IgG fraction of the mouse antiserum (isolated by passage of the serum through Sephadex G-200) could, however, be fully accounted for by its content of each IgG isotype (determined by radioimmunoassay) using the specific haemolytic activities calculated above.

As the IgG1 was unaggregated, activation of C by the alternative pathway seemed unlikely. To assess whether the observed haemolysis resulted from activation of C by the classical pathway, the effect of EDTA or EGTA on lysis by each type of IgG was determined. EGTA blocks classical pathway activation but has little effect on the alternative pathway, whereas EDTA inhibits both routes[12]. The results (Table 2) demonstrate clearly that haemolysis by each IgG isotype was mediated entirely by classical pathway activation. Similar results, including those for IgG1, were obtained when normal mouse serum rather than guinea pig serum was used as the source of C.

These findings are apparently in conflict with present opinion[2,6–8] that mouse IgG1 is a 'non-C-fixing' class of immunoglobulin. In other experiments[13], however, we have isolated IgG1 from the sera of mice immunised with several different antigens and have shown that, in certain instances, IgG1 antibodies are unable to fix C and can effectively block lysis of target cells by C-fixing antibodies through competition for antigenic sites on the target cell surface. Since both types of IgG1 antibody can be produced by inbred strains of mice[14], the genes coding for each type of γ chain cannot be allelic. The C-fixing IgG1 therefore seems to represent a previously undescribed mouse immunoglobulin isotype. We propose that the 'non-C-fixing' and C-fixing IgG1 be termed IgG1a and IgG1b respectively, according to the nomenclature for mouse IgG isotypes based on Fahey *et al.*[1,6]. Whether these functionally distinct types of IgG1 correspond to the two antigenically distinct IgG1 myeloma proteins described by Stanislawski and Mitard[15] remains to be shown.

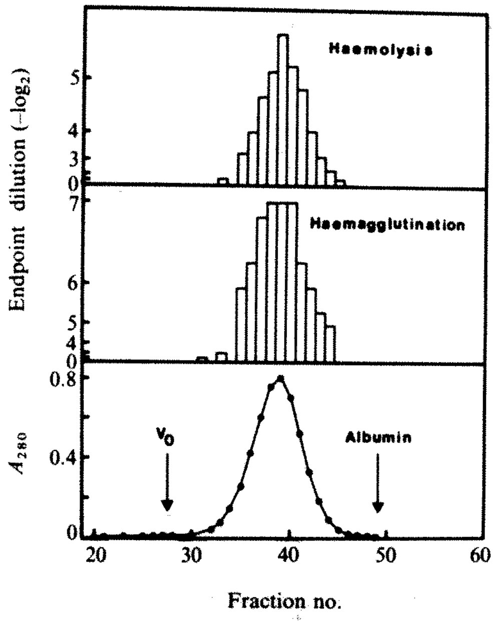

Fig. 2 Sephadex G-200 chromatography of purified IgG1 immunoglobulin. Column size: 2.2×90 cm; sample: 4.1 ml (14.4 mg IgG1); eluant: phosphate-buffered saline (*p*H 7.4) containing 7.5 mM NaN$_3$; flow rate: 15.3 ml h^{-1}. Each fraction (4.1 ml) was assayed for protein (A_{280}) and for SRBC haemagglutinating and haemolytic antibody. A trace of ^{125}I-labelled BSA was added to the sample as an intrinsic marker. The elution positions of albumin and of excluded macromolecules (V_0), determined separately using Blue Dextran 2000 (Pharmacia), are indicated.

Table 2 Haemolytic activity of IgG antibodies in the presence of EGTA or EDTA (% lysis)

Sensitising agent	Control	EGTA	EDTA
IgG1	96.0	2.7	3.7
IgG2a	95.1	3.1	3.5
IgG2b	94.5	4.1	3.3
Inulin	65.2	48.0	2.9

Each tube contained four 50% lytic units of IgG or 5 mg of inulin (Merck). Inulin activates C by the alternative pathway, mediating 'bystander cell lysis' (ref. 17), and was included as a control for alternative pathway activation. The assay diluent was VB^{2+}–BSA with EGTA or EDTA (final concentration, 10 mM) added as indicated. The assay was as described in Fig. 1 except 0.2 ml of guinea pig serum (diluted 1 : 2 in the appropriate diluent) was used. The values shown are the average of triplicate tubes.

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Removal of ambient K^+ inhibits net Na^+ transport in toad bladder by reducing Na^+ permeability of the luminal border

Herbert S. Chase Jr & Qais Al-Awqati

Departments of Medicine and Physiology, Columbia University College of Physicians & Surgeons, New York, New York 10032

Sodium crosses epithelia in two steps: an influx into the cell through the luminal membrane down an electrochemical gradient, and an energy-requiring efflux across the serosal border against a gradient. Koefoed-Johnsen and Ussing[1] originally proposed that cellular efflux of Na^+ at the serosal border of frog skin is coupled to K^+ influx by an exchange pump. It was, therefore, assumed that the inhibition of active Na^+ transport following the removal of K^+ from the serosal side was due to a reduced pool of K^+ available for the coupled exchange. Indirect evidence suggests that this is not correct. (1) K^+ influx from the serosa does not correlate to any significant degree with active Na^+ transport[2–5]. (2) Removal of Na^+ as well as K^+ from the serosal medium substantially reduces the inhibitory effect of K^+ removal on active transport[2,6]. (3) One would predict that if K^+ removal did inhibit active transport only at the serosal pump site, then as active transport decreased the 'active transport pool', that is, the mucosally derived cell Na^+, would increase as it does with ouabain. Studies by Robinson and Macknight[7], however, showed that the mucosally derived pool does not change on removal of external K^+. Essig and Leaf[2] suggested that removal of serosal K^+ inhibited Na^+ transport by decreasing the permeability of the luminal membrane. They showed that when K^+ was removed from the serosa, there was a decrease in the amount of ^{22}Na taken up into the tissue after exposure of the luminal surface to the isotope for 5 min. We have recently found that tissue uptake of ^{22}Na after exposure of toad bladders to the isotope is linear with time for only up to 24 s. Because only the linear portion of the uptake is a measure of the Na^+ flux across the luminal border[8], it is clear that longer periods of uptake are more representative of the steady-state isotope distribution in the tissue. This value is controlled not only by the luminal permeability but also by the serosal permeability and the rate of net transport. These issues prompted us to re-investigate the effect of low external K^+ on Na^+ movement across the luminal border. We report here that the Na^+ transport inhibition seems to be due to a reduced Na^+ permeability at the luminal border.

Toad bladders were exposed to ^{22}Na on the mucosal side for 15 s using Thompson and Dawson's modification[9] of the method of Biber and Curran[10]. The uptake, corrected for extracellular contamination (we shall call this the influx) is determined by two factors, the permeability of the luminal border to Na^+ and the electrical driving force across that membrane.

The Na^+ influx was identical to the simultaneously measured rate of net Na^+ transport evaluated as the short circuit current (Table 1). That the influx is actually a measure of Na^+ movement across the luminal border rather than a measure of Na^+ transport across the whole tissue is seen in experiments using amiloride and ouabain. Although these agents reduced net Na^+ transport equally they affected the influx differently. Amiloride, acting on the mucosal side, abolished tranport and influx. Ouabain, however, inhibited net Na^+ transport by 90% but reduced influx by only 40%.

When K^+ was removed from the serosal side the short-circuit current declined to 50% of its initial value in 20 min. Measurement of the influx at that time revealed that it was reduced by an equivalent amount (Table 1 and Fig. 1). As the influx is the product of the Na^+ permeability of the apical membrane and the electrical driving force across that membrane, the cause of the decrease could be due to either factor. Recent studies by deLong and Civan[5], however, have shown that removal of K^+ had no effect on the cell potential in toad bladder. Further, as can be seen in Fig. 1, the influx, in some instances, was reduced to near-zero levels, suggesting that if the only cause for the reduction was a change in the potential, the intracellular voltage must have attained a very large positive value. These considerations suggest that the permeability of the luminal border decreased to cause a reduction in the influx and consequently the short-circuit current.

The identity between influx and short circuit current indicates that the reduction in net transport is due to a reduced Na^+ entry

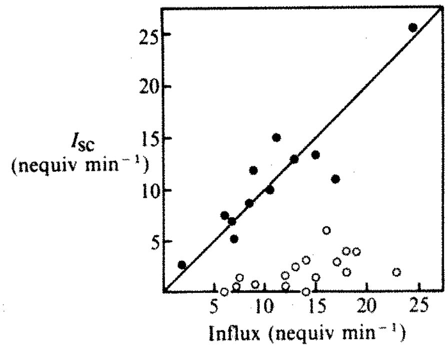

Fig. 1 Plot of the simultaneously measured short circuit current (I_{sc}) with respect to the influx (J) after 20 min of either ouabain (1.0 mM, ○) or K^+-free (●) inhibition. The solid line represents the identity between the short circuit current and the influx obtained in control bladders. The line describing the K^+-free points is $I_{sc} = 0.86\ (\pm0.14)J + 1.5$ nequiv. min^{-1} ($r = 0.89$) and that of the ouabain points is $I_{sc} = 0.22\ (\pm0.08)J - 0.79$ nequiv. min^{-1} ($r = 0.60$). Mucosal Na^+ concentration was 12 mM in all experiments.

Table 1 Effect of ouabain and K^+-free serosal solution on short circuit current and influx

Serosal solution changed to	Initial I_{sc} (nequiv. min^{-1})	Following serosal change I_{sc}	Following serosal change Influx
Ringer's (control)	19.5	21.3	20.6
($n = 9$)	±2.1	±2.8	±3.1
Ouabain (1.0 mM)	24.0	2.2	14.0
($n = 16$)	±1.6	±0.4	±1.2
K^+-Free	22.9	11.0	10.9
($n = 12$)	±1.6	±1.7	±1.8
	Initial I_{sc}	Amiloride I_{sc}	Amiloride Influx
Ringer's	21.1	0	0.1
($n = 4$)	±1.9		±0.1
Ouabain	20.4	0	−0.2
($n = 4$)	±0.9		±0.3
K^+-Free	19.1	0	0.1
($n = 3$)	±7.9		±0.2

In the above experiments small pieces of toad bladder (1.5 cm^2) were mounted in modified Ussing chambers and short-circuited until the current reached a steady value (initial I_{sc}). For all experiments the mucosal solution consisted of (in mM) NaCl 10, $NaHCO_3$ 2.5, KCl 3.5, $CaCl_2$ 1.0 and choline chloride 100. The serosal buffer contained NaCl 110, $NaHCO_3$ 2.5, KCl 3.5 and $CaCl_2$ 1.0. The KCl was omitted without substitution in the K^+-free studies. After the I_{sc} had reached a steady level the serosal solutions were changed. Twenty minutes later the I_{sc} was recorded while the ^{22}Na uptake was being studied. For the control bladders the slope of the influx as a function of short circuit current was 0.95 ± 0.24, intercept 0.5 ± 5.6 nequiv. min^{-1}, $r = 0.83$. In a larger series of control bladders ($n = 20$), influx $= 0.97(\pm0.14) + 0.03(\pm1.10)$ nequiv. min^{-1}, $r = 0.86$. In a separate experiment amiloride (0.1 mM) was added to the mucosa 20 min after the same serosal changes made in the previous experiment.

into the cell. However, these results do not rule out other effects of K^+-free media on the Na^+ pump. Figure 2 shows that the inhibition of the luminal permeability is the major cause of the reduction in net transport. Amphotericin B, when added to the mucosal medium, induces channels in the luminal membrane which accelerate Na^+ entry into the cell[11]. When removal of external K^+ reduced the short circuit current to 30% of its initial value, mucosal addition of 15 $\mu g\,ml^{-1}$ of amphotericin B increased the short circuit current, restoring it to near the initial rate. Ouabain completely inhibited this new current, indicating the current was due to the activity of the Na^+ pump. These results show that the inhibition of net transport by removal of external K^+ was due largely to the reduction of the luminal permeability to Na^+.

As the Na^+ pump is probably the $(Na^+ + K^+)$ATPase, it is rather puzzling that amphotericin enhanced net transport in the absence of external K^+. The most probable explanation is that, although there is no K^+ in the bulk solution, it is unlikely that the K^+ concentration in the unstirred layer next to the serosal border is actually zero. Hence, the pump may still be operating in the Na^+,K^+ mode. Alternatively, it may be exchanging Na^+ for Na^+ in an 'electrogenic' manner.

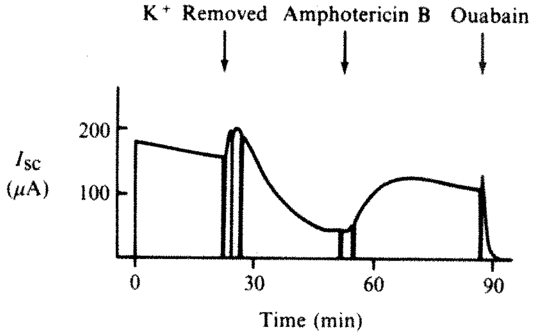

Fig. 2 The tracing obtained from a toad hemibladder mounted in a standard Ussing chamber. As marked, after an initial period during which the bladder reached a steady value, the serosal K^+ was removed (the solution was changed three times). After 20 min, when the current had fallen to ~30% of the initial value, amphotericin B (15 $\mu g\,ml^{-1}$) was added to the mucosal side. Thirty minutes later ouabain (1 mM) was added to the serosal bath in the presence of mucosal amphotericin. The average values of the seven experiments carried out were (in μA): initial I_{sc}, 175 ± 24; 20 min after serosal K^+ removal, 68 ± 13; peak response to amphotericin, 129 ± 19; after ouabain, 0. The mucosal Na^+ concentration was 12 mM.

When serosal K^+ is removed the cells lose K^+ and gain Na^+ concomitantly with the decline in Na^+ transport[2,7]. Removal of serosal Na^+ and K^+ prevents the changes in cell ionic composition and the fall in active transport. These data suggest that changes in intracellular ionic composition are the cause of the reduced permeability of the luminal membrane. The increase in cell Na^+ concentration could reduce the permeability of the Na^+ channels by an allosteric mechanism similar to that invoked by Lindemann[12] and Cuthbert[13] to explain the effect of external Na^+ concentration on the Na^+ permeability of the luminal membrane. Alternatively, an increase in cell Na^+ might increase cytosolic Ca^{2+} (ref. 14) through $Na^+:Ca^{2+}$ exchange across cell and mitochondrial membranes. As with Na^+, external Ca^{2+} has been shown to reduce the Na^+ permeability of the apical membrane[15]. Whether any of these or other mechanisms are involved must be determined by future studies.

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Direct addition of insulin inhibits a high affinity Ca^{2+}-ATPase in isolated adipocyte plasma membranes

Harrihar A. Pershadsingh & Jay M. McDonald

Division of Laboratory Medicine, Departments of Pathology and Medicine, Washington University School of Medicine and Barnes Hospital, St Louis, Missouri 63110

The mechanism by which insulin regulates cellular metabolism remains unknown although indirect evidence suggests that alterations in intracellular calcium are important. More specifically, it has been proposed that insulin triggers an increase in intracellular calcium which is responsible for the subsequent modification of metabolic activities[1,2]. The cell maintains a large electrochemical gradient for ionised calcium between the cytoplasm ($<10^{-6}$ M, as determined for muscle[3] and nerve[4]) and the extracellular environment ($>10^{-3}$ M). The plasma membrane may, therefore, be important in the regulation of calcium homeostasis, as a slight alteration in the processes maintaining this gradient could result in marked changes in cytoplasmic calcium. One such process is the active extrusion of calcium from the cell by a high affinity calcium-stimulated ATPase (Ca^{2+}-ATPase). Such a mechanism has been well established in red cells [5] and is postulated in nerve[6], liver[7] and muscle[8]. We have identified a high affinity Ca^{2+}-ATPase in a plasma membrane-enriched subcellular fraction isolated from rat adipocytes[9] which may provide the enzymatic basis for a calcium extrusion pump. We demonstrate here that the Ca^{2+}-ATPase is specifically inhibited by the direct addition of physiological concentrations of insulin to the isolated plasma membranes. This effect suggests that direct regulation of calcium homeostasis may represent an important event in the mechanism of action of insulin.

The high affinity Ca^{2+}-ATPase of rat adipocyte plasma membranes had an apparent half saturation constant ($K_{0.5}$) of 0.14 ± 0.04 μM (mean ± s.e.m. of eight experiments as in Fig. 1) for ionised calcium. A Hill plot of the data in Fig. 1 yielded a straight line with a Hill number of 1.5 (Fig. 1 inset). The maximal activity of the high affinity Ca^{2+}-ATPase was 196 ± 41 nmol $^{32}P\,mg^{-1}\,min^{-1}$ (mean ± s.e.m., five experiments) in membranes stored for less than two days. Activity decreased markedly after longer storage. Another saturable component appeared at higher calcium concentrations ($K_{0.5} \simeq 74$ μM), indicating the presence of a low affinity site similar to that previously reported for this tissue[10]. The high affinity enzyme yielded a biphasic response to varying ATP concentrations, also representing the sum of two saturable components, one with $K_{0.5} \simeq 0.8$ μM, the other with $K_{0.5} \simeq 27$ μM (data not shown). The presence of 20 mM sodium, 20 mM potassium or 0.5 mM ouabain did not affect the Ca^{2+}-ATPase, but activity was completely inhibited by 0.2 mM lanthanum. The enzyme had an absolute requirement for the endogeneous magnesium that was present in the plasma membrane preparation. Chelation of magnesium by *trans*-cyclohexane-1,2-diamine-*N*, *N*, *N'*, *N'*-tetraacetic acid

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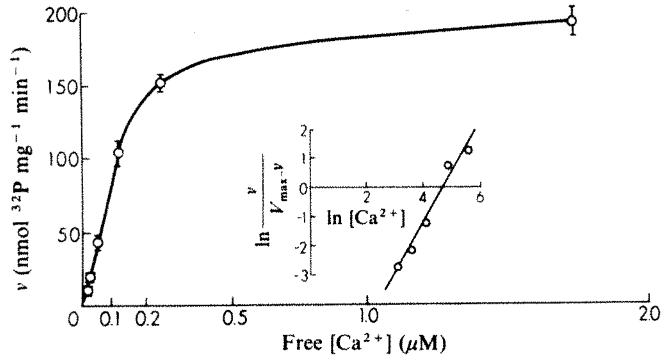

Fig. 1 Calcium dependence of the high affinity Ca^{2+}-ATPase in adipocyte plasma membranes. Isolated adipocytes were prepared from the epididymal fat pads of 120 g Sprague–Dawley rats[35] and the plasma membrane fraction obtained by a modification of the method of McKeel and Jarett[11] in which EDTA was omitted from the fractionation and homogenisation media[36]. The membrane fraction used in these studies has been previously characterised and has been shown to be highly enriched in plasma membranes by the use of the specific marker enzymes adenylate cyclase and 5′-nucleotidase[37]. The distribution of the high affinity Ca^{2+}-ATPase was shown to parallel the distribution of 5′-nucleotidase among the subcellular fractions (unpublished observations). These plasma membranes, which specifically bind ^{125}I-insulin[38], were either used immediately or within two days after storage at −70 °C. Ca^{2+}-ATPase activity was quantitated by monitoring ^{32}P released from γ ^{32}P-ATP and is expressed as nmol ^{32}P per mg protein per min[39]. Protein values were obtained by a previously described method[40]. The assay medium contained 20 μg protein ml^{-1} with 200 μM EGTA, 100–200 μM $CaCl_2$ (equivalent to 0.02–1.80 μM free Ca^{2+}), 1 mM Na_2ATP, 20 mM Na azide and 12.5 mM Tris-PIPES, *p*H 7.5 at 37 °C. Endogenous calcium in the assay medium was determined to be 4.5–5.0 μM by analysing the stock reagent solutions used by atomic absorption spectroscopy[41]. The total calcium concentrations used to calculate the ionised component take into account the endogeneous calcium present in the reagents. Ca^{2+}-ATPase activity was determined by subtracting values obtained with EGTA alone from those obtained with calcium plus EGTA. Each point represents the mean ±s.e.m. of quadruplicate determinations. Inset: Hill plot of the data. Free [Ca^{2+}] was calculated using a Ca-EGTA apparent association constant of $10^{7.832}$ M^{-1} whch was determined for *p*H 7.5 as described by Portzehl *et al.*[3]. The concentration for ionised calcium was corrected for any effect of ATP using the equations described by Portzehl *et al.*[3] and Schatzmann[42] and a Ca-ATP apparent association constant of $10^{3.930}$ M^{-1}, calculated at *p*H 7.5. All cation–ligand association constants were obtained from Sillén and Martell[43].

(CDTA), which has a high affinity for magnesium, totally abolished Ca^{2+}-ATPase activity. Activity could be fully restored by the addition of micromolar concentrations of free magnesium.

The high affinity Ca^{2+}-ATPase was located predominantly in the plasma membranes. This was determined by assessing its relative distribution among subcellular membrane fractions. The specific Ca^{2+}-ATPase activities in mitochondrial and microsomal (enriched in endoplasmic reticulum) fractions were 10 and 32%, respectively, of that of the plasma membrane fraction and could be accounted for by plasma membrane contamination[11,12].

Direct addition of insulin to the plasma membranes resulted in a highly significant decrease in Ca^{2+}-ATPase activity at various calcium concentrations in the region of the $K_{0.5}$ for calcium (Table 1). The per cent inhibition by insulin was greatest at the lowest calcium concentration and became less at higher concentrations, suggesting that insulin interaction with the plasma membranes may result primarily in a decreased affinity of the enzyme for calcium. The specificity of the insulin effect was shown in two ways. First, the inhibitory effect was concentration dependent over the physiological range for insulin (Fig. 2), increasing sharply between 0 and 50 μU insulin ml^{-1} and plateauing between 50 and 100 μU ml^{-1}. This concentration dependence is similar to other physiological responses to insulin such as glucose transport and oxidation[13]. Second, direct addition of equimolar concentrations of either desoctapeptide insulin, an analogue which has ~1% of the biological activity of insulin, or insulin which had been inactivated by boiling for 15 min did not inhibit the Ca^{2+}-ATPase. A similar inhibition was observed in plasma membranes prepared from cells which were pretreated with 100 μU insulin ml^{-1} at 37 °C for 15 min compared to plasma membranes simultaneously prepared from control cells[14] (data not shown). As the same effect is also seen in the intact cell, this similarity suggests that inhibition of the Ca^{2+}-ATPase by the direct addition of insulin to the plasma membranes is not an artefact.

The characteristics of the high affinity Ca^{2+}-ATPase in adipocyte plasma membranes strongly suggest that it may provide the enzymatic basis for a pump which extrudes calcium from the cell analogous to the well-characterised calcium transport enzyme in red cell plasma membranes[5]. Both enzymes are similar in that they: (1) require magnesium, (2) possess a $K_{0.5}$ for calcium close to the concentration of ionised calcium in the cytoplasm, (3) have similar low and high affinities for ATP, (4) are relatively independent of sodium or potassium ions, (5) are insensitive to ouabain, (6) are completely inhibited by lanthanum, and (7) possess a Hill number greater than one and less than two[5,15,16]. It is not surprising that such a calcium extrusion pump may exist in adipocytes as its presence has previously been suggested from calcium flux studies using intact cells[17].

An insulin-induced inhibition of calcium extrusion via inhibition of a high affinity pump enzyme could be a critical event in the mechanism of insulin action. Several observations may be related to this proposal. First, pretreatment of cells with insulin results in an increase in calcium bound to subsequently isolated adipocyte plasma membranes[18] and skeletal muscle sarcolemma[19]. Increased cytoplasmic calcium resulting from the inhibition of a calcium pump may regulate the availability of calcium for binding to specific sites on the plasma membrane. This increase in bound calcium could be responsible for the known effect of insulin to hyperpolarise muscle[20] and fat cells[21], as calcium has been shown to influence membrane permeability[22,23] and the membrane potential[24-26] of other electrically inexcitable cells. Second, the observations reported here could

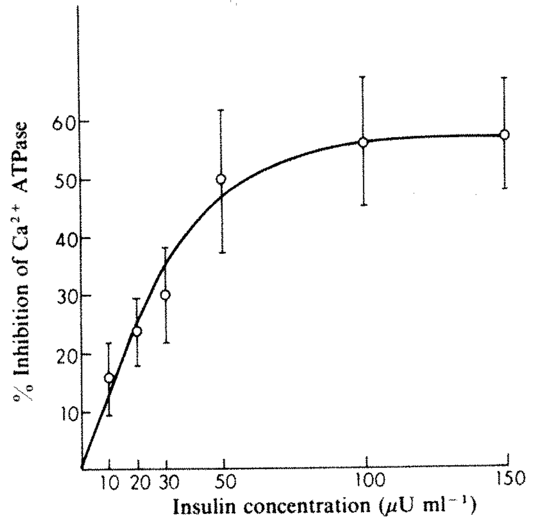

Fig. 2 Concentration-dependent inhibition of the high affinity Ca^{2+}-ATPase by insulin. Assay conditions were identical to those described in Fig. 1 and Table 1. Each point represents the mean ± s.e.m. of results obtained from four different membrane preparations. Free [Ca^{2+}] is 3.5×10^{-8} M.

also relate to insulin's regulation of glucose transport and membrane phosphorylation. Both membrane-bound calcium[27] and membrane phosphorylation[28] have been implicated in the regulation of glucose transport, and ATP has been reported to have a permissive effect on insulin-stimulated glucose transport[28]. Also, direct addition of insulin to these adipocyte plasma membranes has recently been shown to specifically inhibit the phosphorylation of two protein bands[29]. One is a 120,000 molecular weight (MW) plasma membrane protein; the other a 42,000-MW protein which originates from the small amount of mitochondria present in the preparation and has been identified as the α subunit of pyruvate dehydrogenase, an insulin-sensitive enzyme[29]. The unidentified insulin-sensitive 120,000 MW plasma membrane protein could represent the high affinity Ca^{2+}-ATPase as the analogous red cell Ca^{2+}-ATPase phosphoprotein has a similar molecular weight[30].

Table 1 Inhibitory effect of insulin on Ca^{2+}-ATPase activity at different free calcium concentrations*

	Ca^{2+}-ATPase activity ($nmol\ ^{32}P\ mg^{-1}\ min^{-1}$)			
Free $[Ca^{2+}]$	Control	+Insulin	% Inhibition	Significance
4.2×10^{-8} M	28.7 ± 6.7	16.9 ± 4.4	43.1 ± 7.7	$P<0.01$ ($n=9$)
7.1×10^{-8} M	49.6 ± 8.0	38.4 ± 7.3	25.1 ± 9.8	$P<0.02$ ($n=8$)
1.6×10^{-7} M	104.1 ± 19.1	92.4 ± 17.5	13.3 ± 3.1	$P<0.01$ ($n=7$)

* Assay conditions were identical to those in Fig. 1. Insulin was added to a final concentration of 100 μU ml^{-1} from a stock solution containing 0.1% bovine serum albumin (BSA). The final BSA concentration in each assay was 0.00125%. Controls contained an identical concentration of BSA. Results are expressed as the mean ± s.e.m. of *n* different membrane preparations. Significance was determined using the two-tailed paired *T*-test.

Increased cytoplasmic calcium resulting from inhibition of a calcium extrusion pump could directly regulate a variety of insulin-sensitive calcium-dependent enzymes including glycogen synthetase[31], hormone-sensitive lipase[32] and pyruvate dehydrogenase[33]. Alternatively, this increased calcium could mediate a variety of intracellular processes via binding to a calcium-dependent regulatory protein, which has recently been identified in the cytoplasm of adipocytes[34].

Thus these data demonstrate a direct effect of insulin on a high affinity Ca^{2+}-ATPase in the plasma membrane. Although further work is necessary to elucidate its role in insulin action and the mechanism whereby insulin inhibits this enzyme, our observations imply an important and fundamental role of calcium in the mechanism of insulin action.

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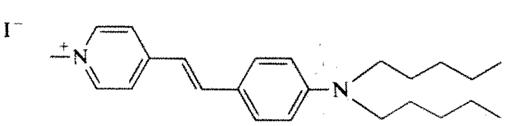
Evidence for a charge-shift electrochromic mechanism in a probe of membrane potential

L. M. Loew*, S. Scully*, L. Simpson* & A. S. Waggoner†

* Department of Chemistry, State University of New York at
at Binghamton, Binghamton, New York 13901
† Department of Chemistry, Amherst College, Amherst,
Massachusetts 01002

Extrinsic optical probes have become important tools for monitoring membrane potential, with probes now available for many tissue or cell suspension systems[1]. In each case that has been studied in detail[2–4], it seems that the mechanism involves a shift in the equilibrium population of the probe from one chemical environment to another in response to the transmembrane potential; the environments perturb the probe's spectrum differently. As this indirect mechanism involves a redistribution of dye between chemical environments that are likely to vary if a given probe is transferred from one membrane to another, a potential probe that is effective and calibrated for all membrane systems has not been realised. We present here evidence for a direct response of a probe chromophore to the electric field across membrane systems. The results suggest it might be possible to develop a universal set of membrane probes.

The probe used is the first to emerge[5] from a recently developed theoretical methodology[6]. According to this theory, the probe, 4-[*p*-(dipentylamino)styryl]-1-methyl-pyridinium iodide (di-5-ASP), has a ground state charge distribution which



favours orientation of the pyridinium ring near the aqueous interface, with the probe long axis orientated perpendicular to the membrane surface. The theory also predicts that, upon photoexcitation, the centre of positive charge will undergo a shift from the pyridinium end to the aminophenyl end of the chromophore. A transmembrane electric field should therefore induce a direct electrochromic shift[7,8] of the absorption spectrum of di-5-ASP[6].

The spectral responses of di-5-ASP bound to a hemispherical bilayer membrane of oxidised cholesterol[9] were used to provide experimental evidence for this predicted mechanism. The apparatus for determination of potential dependent fluorescence changes is shown in Fig. 1. Absorbance changes were determined with an apparatus essentially identical to that previously described for experiments with a planar bilayer[3].

An important criterion for electrochromism is that the first derivative of the absorption (or excitation) spectrum closely matches the response spectrum[10]. Application of this criterion to di-5-ASP is illustrated in Fig. 2; there is a small deviation,

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probably because the most responsive probe molecules are those orientated perpendicular to the membrane and these, in turn, have shorter wavelength absorption[11]. Figure 2 also illustrates how the probe senses the reversed potential when it is applied to the inside of the membrane. Hyperpolarisation of the membrane causes a blueshift of the spectrum when probe is applied to the inside and a redshift for probe bound to the outer surface, consistent with the behaviour predicted for a charge shift electrochromic mechanism[6]. On the other hand, an 'on-off' mechanism[3] would predict the opposite behaviour, as membrane bound di-5-ASP is blueshifted with respect to aqueous di-5-ASP[11].

The orientation dependence alluded to above is demonstrated in Fig. 3. The probe has a strong tendency to align perpendicular to the membrane surface[11], a feature which was an important design consideration. Fig. 3 shows that the response to potential is even more sensitive to orientation, showing a dichroism of ~80:1 at 480 nm. This can be easily rationalised in terms of electrochromism, as the small population of probes capable of absorbing light polarised parallel to the membrane would also have a relatively lower interaction with the transmembrane electric field.

A true electrochromic response is instantaneous on the time scale of any event which requires movement of nuclei, such as the development of a membrane potential. We have determined that, within the admittedly slow time scale required for charging of the hemispherical bilayer (100 μs), this condition is met for the absorption response of di-5-ASP.

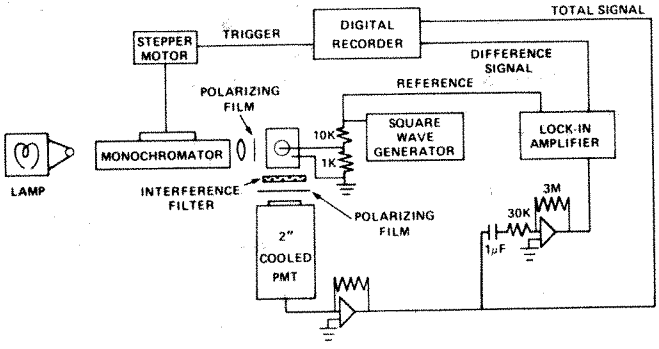

Fig. 1 System for the determination of corrected excitation and potential response spectra from probe-labelled hemispherical lipid bilayers. [System components are: Oriel 100-W quartz-halogen source, powered by Sorensen d.c. supply (DCR40-25B); Instruments SA model H20V monochromator with stepping motor and controller; 570 nm 2×2 inch interference filter (Ditric Optics); EMI-9558QA photomultiplier in Pacific Photometric Instruments model 3463 thermoelectrically cooled housing; Princeton Applied Research 5203 lockin amplifier-tuned amplifier; B&K Precision square wave generator; Bascom Turner 8110 recorder.] A decane solution of oxidised cholesterol[9] was used to prepare hemispherical bilayers. The solution was lightly brushed along the bottom of a 100 mM KCl solution contained in a 2 mm i.d. polyethylene tube which was attached to a micrometer syringe. The tube was immersed in 100 mM KCl contained in a cuvette, and a hemispherical bilayer was formed via gentle pressure from the syringe[9]. The square-wave potential pulses were applied through Ag-AgCl electrodes in the tube and the cuvette. The appropriate quantity of probe was added to the aqueous KCl as a 3 mM solution in ethanol; the total ethanol concentration was always below 0.3%. (Probe synthesis as in ref. 11). Since membrane-bound di-5-ASP fluoresces ~100 times more strongly than aqueous di-5-ASP, fluorescence detection is an effective way of isolating the membrane response; also, the emission interference filter rejects aqueous fluorescence ($\lambda_{max} = 620$ nm) but passes membrane fluorescence ($\lambda_{max} = 575$ nm). One of the key components of the system is the digital recorder which allows simultaneous acquisition of response spectra and excitation spectra. A rhodamine B quantum counter was used to generate a calibration curve, stored on the recorder's floppy disk, to correct the experimental spectra for the wavelength dependence of the exciting light intensity.

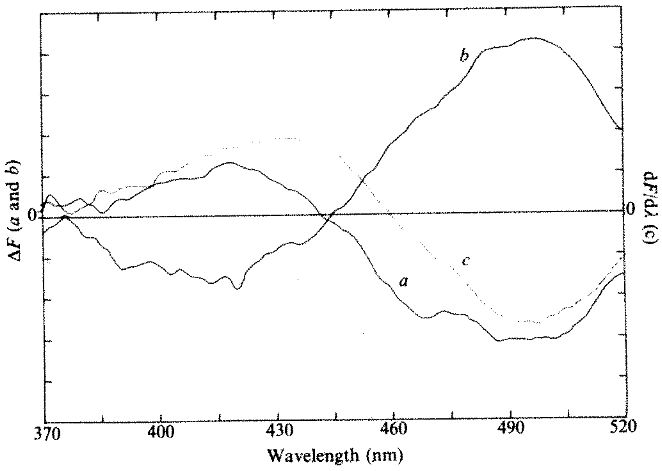

Fig. 2 Corrected response of the probe excitation spectrum to a 100 mV potential pulsed at 200 Hz. Apparatus described in Fig. 1. *a*, 3 μM probe applied outside the bubble; *b*, 3 μM probe inside; *c*, first derivative of the corrected excitation spectrum.

In addition to providing positive evidence for charge-shift electrochromism, the experimental results argue against the more common mechanisms[2-4] adopted by potential probes. At low lipid : probe ratios, aggregation of di-5-ASP can be observed in the membrane, detected by a blueshift in the absorbance and a quenching of fluorescence (L. M. L. and L. S., unpublished results); the similarity of the absorption response (Fig. 3) and the excitation response (Fig. 2) spectra, rule out the involvement of these non-fluorescing aggregates in the mechanism. A reorientation mechanism[12] is precluded by the results of Fig. 3; such a mechanism would require responses of opposite sign for the two polarisations. Another possible, and in this case unprecedented, process involves electrophoretic movement of the probe from one membrane site to another, with the two environments sufficiently different to induce a spectral shift. In some recent experiments[13] we have found essentially identical spectra with an electrically neutral analogue of di-5-ASP; this is strong evidence against the involvement of simple electrophoresis in the mechanism.

Electrochromism will assure a fast, well characterised and, perhaps, uniform response to membrane potential which may provide a special niche for di-5-ASP within the large repertoire of optical probes. Some of its other characteristics will be equally important in determining its usefulness, however. As $\Delta F/F$ is as

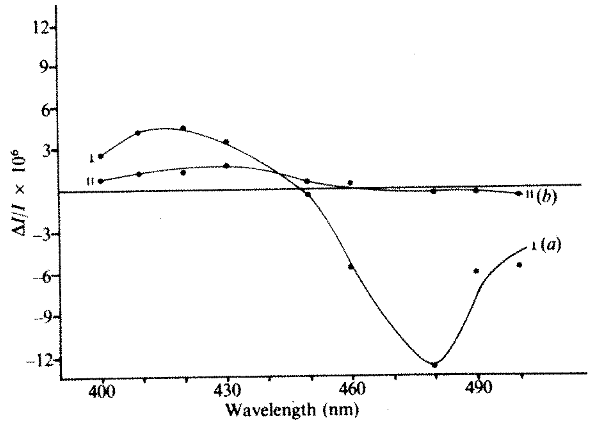

Fig. 3 Response of the absorption spectrum of 5 μM probe to 100 mV potential steps applied across the hemispherical membrane at 200 Hz. *a*, Incident light focused on the bottom $\frac{1}{8}$ of the bilayer and polarised perpendicular to this approximately planar area; *b*, polarisation parallel to the membrane. The apparatus has been previously described[3].

large as 5% for a 100 mV change in potential when exciting near the red wing of the absorption band, it is likely that monitoring fluorescence will provide the best signal. The probe binds strongly to membranes (in lipid vesicle experiments[11] we were unable to detect unbound probe even at a 1 : 1 lipid : probe ratio). The membrane-probe fluorescence is blueshifted by ~40 nm (to 575 nm) and is ~100 times more intense relative to aqueous probe; this provides obvious practical advantages in some applications, but would lower the relative change in fluorescence in an axon experiment, for example, as a result of high background fluorescence from the Schwann cells. The photostability of di-5-ASP appears good, a 3 mM ethanol solution having survived 2 years on an open shelf, so that high intensity excitation could be used to reduce shot noise; the question of photodynamic damage, however, is still open.

Di-5-ASP was the first 'charge-shift' probe to be examined, as much because of its simple preparation as its theoretical promise. The results presented here suggest that it will be worthwhile to develop new charge shift probes which may fit other spectral windows and be even more sensitive. The operation of these probes does not involve a delicate field-dependent balance between different chemical environments, so this approach may provide a universal set of spectroscopic potential probes.

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The molecular structure of lecithin dihydrate

Robert H. Pearson

Medical Foundation of Buffalo and Electron Optics Laboratory, Biophysics Department, Roswell Park Memorial Institute, Buffalo, New York 14263

Irmin Pascher

Department of Structural Chemistry, Göteborg University, POB, S40033 Göteborg, Sweden

Lecithin is a major structural component of biological membranes[1,2]. Because of their amphipathic nature, lecithin and related phospholipids tend to aggregate as bilayer structures in which the hydrophilic head groups are orientated towards the surface and the hydrophobic hydrocarbon chains towards the interior. A detailed knowledge of the three-dimensional structure of lecithins will aid in the understanding of their role in membrane structure and function, but is still lacking. To this end we have now crystallised and solved the molecular structure of 1,2-dimyristoyl-*sn*-glycero-3-phosphorylcholine (DMPC), a lecithin species in the naturally occurring configuration. This compound crystallises from a water-containing solution, with two water molecules (5% w/w) of hydration.

The crystals were grown from commercially available material (Sigma, 98% pure) which had been further purified by recrystallisation from an ether/ethanol/water solution. In this solvent the nucleation of the crystals is accomplished by lowering the temperature of the solution to 14 °C, at which a flocculent precipitate appears. Once nucleated, growth can occur at any temperature between 0 °C and 22 °C, but above 22 °C the crystals redissolve. The crystals are monoclinic, space group $P2_1$, with two molecules in the asymmetric unit. Unit cell parameters are: $a = 8.72$, $b = 8.92$, $c = 55.4$ Å and $\beta = 97.40°$. Reflection data were collected by a peak measurement technique on a GE-XRD6 manual diffractometer. Of the 4,800 reflections thus collected, 1,931 were considered observable by the 2σ criterion. To maintain the crystals and improve high-angle data quality, the crystals were kept below 20 °C at all times and data were collected at 10–15 °C.

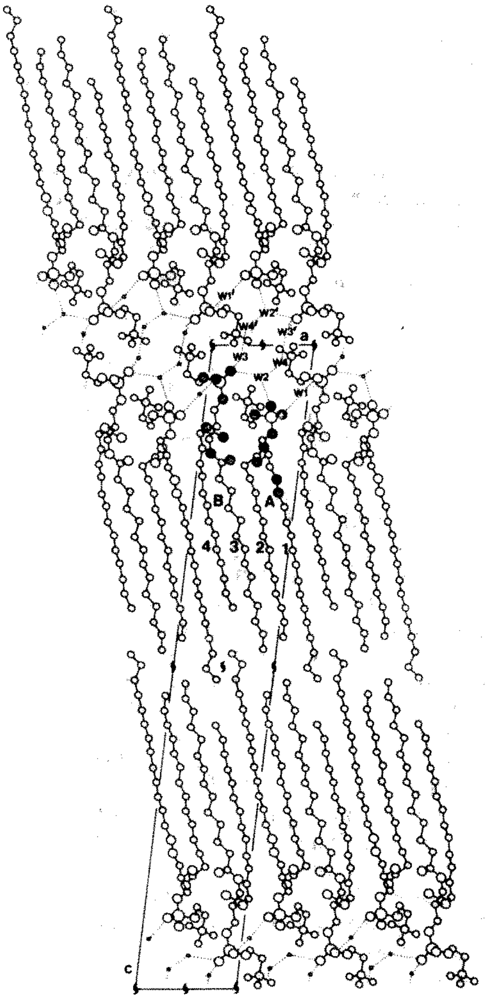

Fig. 1 Molecular arrangement of dimyristoyl lecithin dihydrate projected onto the *ac*-plane. The two molecules A and B of the asymmetric unit are shown with shaded oxygen atoms. The notation of the water molecules of hydration is given for a representative part of the hydrogen bond system (dotted lines) at the polar bilayer interface. The position of the remaining water molecules is indicated by small circles only. The break in the hydrogen bonds at W3' is intended to convey the helical nature of the water structure; connection of this bond leads to the phosphate oxygen of one unit below.

The structure was solved by Patterson and Fourier techniques. The positions of the two phosphorus atoms were found in the Harker sections of the Patterson map and difference Patterson map (where the contribution of the chain carbons had been removed). The remainder of the two molecules in the asymmetric unit was found in successive Fourier maps. Least-squares refinement with isotropic temperature factors (using the Nonius CAD-4 software system for the PDP-11 computer) has reduced the residual factor to 17.4%. The refinement procedure is being continued. An increase in the number of parameters by introducing anisotropic temperature factors is, however, not considered compatible with the number of observed reflections.

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The molecules are arranged in a bilayer structure with strong hydrogen bonding in the polar portions of the crystal (Fig. 1). The two molecules (A and B) in the asymmetric part of the unit cell are displaced from one another in the direction of the bilayer normal by 2.5 Å, or one whole zigzag unit of a hydrocarbon chain. Due to this displacement the bulky phosphorylcholine groups require a rather small area (38.9 Å^2 per molecule) of the layer surface which allows the two hydrocarbon chains to be almost perpendicular (78°) to the bilayer boundary. This type of offset may be related to the displacement of molecules observed in the rippled $P\beta'$ structure of lecithin bilayers in more fully hydrated conditions[3,4]. Phosphatidylethanolamine (PE), which has a smaller polar group, does not form such rippled bilayers. In the crystal structure of 1,2-dilauroyl-DL-phosphatidylethanolamine (DLPE)[5,6] the molecules occupy practically the same area of 38.6 Å^2 in the layer plane but align with their polar groups parallel to the bilayer surface, without a mutual displacement in direction of the bilayer normal. A corresponding arrangement of phosphorylcholine groups which is observed in the crystal structure of the lysolecithin analogue, 1-lauroyl-propandiol-3-phosphorylcholine monohydrate (LPPC)[7], requires an area per molecule of 52.1 Å^2. Although there are many common features in the conformation of the molecules in the crystal structure of DLPE and DMPC, a significant difference exists with respect to the interaction between the polar groups. In DLPE the $-NH_3^+$ group forms superimposed hydrogen bonds/salt bridges 2.8 Å long with unesterified phosphate oxygens of adjacent molecules. In DMPC (and LPPC) the nitrogen atom of the bulky $-N(CH_3)_3^+$ group comes no closer to the phosphate oxygens than 4.5 (3.7) Å. Instead, the non-ester oxygens which share the negative charge are separated and shielded by water molecules of hydration. Two water molecules (W1 and W2) are located between the phosphate oxygens of molecules A and B and thus form an infinite hydrogen-bonded phosphate (A)–water–phosphate (B)–water ribbon (Fig. 1) which provides stability in the *a*-axis direction of the bilayer plane. A similar phosphate–water ribbon is observed in the crystal structure of LPPC. The two other water molecules (W3 and W4) are hydrogen bonded to one phosphate oxygen of molecule B and to water molecule W2, respectively, and establish a linkage across the bilayer interface with the corresponding symmetry-related water molecules (W3′ and W4′) of the adjacent bilayer. Thus, a helical water structure about the two-fold screw axis is formed (see Fig. 1) which provides structural stability between the bilayers and in the *b*-axis direction of the bilayer plane.

The lateral arrangement of the hydrocarbon matrix is not consistent with earlier known simple chain packing modes. The orientation of the chain planes is alternately parallel and perpendicular to each other and resembles to some extent the hybrid chain packing modes[8] observed for other double chain lipids. The cross-section of 19.0 Å^2 per chain also corresponds to that of cerebroside[9] (19.1 Å^2), whereas DLPE is slightly larger (19.3 Å) due to its shorter chains. Note, however, that the hybrid packing in DMPC is well defined near the carboxyl end only. Towards the methyl end the chain planes are twisted to a mutually parallel orientation resembling a triclinic parallel (T∥) chain packing mode. This successive change in chain orientation indicates the close relationship between this hybrid subcell and the hexagonal chain arrangement with increased rotational disorder, as has been reported for the gel state of fully hydrated bilayers[3,4]. The small chain tilt of 12° which is observed for DMPC, but not for DLPE (0°), is due to the minute differences in molecular area (+0.3 Å^2) and chain packing density (−0.3 Å^2, see above).

Figure 2 is a detailed picture of the molecular conformation of the two DMPC molecules. The main difference between the two molecules concerns the conformation and orientation of their polar groups. The phosphorylcholine chains are almost mirror images of one another as shown by the torsion angles listed in Table 1. These angles are very similar to those observed in the crystal structures of other phospholipids and phospholipid constituents, which are included in Table 1 for comparison. This preferred conformation common to all phosphorylcholine and ethanolamine compounds reflects the tendency of the ammonium nitrogen to fold back towards the phosphate group so as to minimise the distance between the groups of opposite charge.

The phosphorylcholine groups of the two DMPC molecules make small angles with the bilayer surface. The **P–N** vectors are 4.3 and 4.5 Å long and inclined to the plane of the bilayer by 17° and 27° for molecules A and B, respectively. The somewhat more extended conformation of the molecule B polar group apparently arises from its more intimate participation in the water structure and its interaction with charged groups of the adjacent bilayer. This stretching and increased inclination of the polar group (which mainly affects torsion angle α_4 (see Fig. 2 and Table 1)) might also be expected in natural membranes when they become associated with ions. The cadmium chloride complex of GPC[10] listed in Table 1 reflects the response of the polar group conformation to the proximity of metal ions, and NMR studies on lecithin in the presence of polyvalent cations[11] confirm this.

The most obvious conformational difference between the two DMPC molecules, however, is in the orientation of the polar groups with respect to the hydrophobic parts. In molecule A the phosphorylcholine group points away from its own diglyceride part, but is close to it in molecule B. These two orientations are due to different conformations about the $C_{(3)}$–$C_{(2)}$ bond of the glycerol moiety (see Fig. 2 and Table 1). The torsion angle, θ_1, about this bond is +58° (+*gauche*) and +169° (*antiplanar*) in molecules A and B, respectively. In DLPE another staggered conformation ($\theta_1 = -52°$; −*gauche*) is adopted whereas in LPPC an eclipsed *synplanar* conformation ($\theta_1 = 28°$) is approached. This shows that there are no preferred conformations about the $C_{(2)}$–$C_{(3)}$ bond. This bond apparently allows rotational independence between the asymmetric (1,2-*sn*-configuration) and conformationally similar diglyceride moiety (see below) and the polar group which exists as mirror image conformers. Results from deuterium NMR studies[12] indicate that in both the gel and liquid-crystalline states of lecithin, such a rotation of the polar group takes place, and seems to be accompanied by a rapid

Table 1 Torsion angles of crystallographically determined phospholipid constituents*

		α_1	α_2	α_3	α_4	α_5	θ_1	θ_3	β_1	β_2	β_3	β_4	γ_1
1,2-dimyristoyl-*sn*-glycero-3-phosphorylcholine.2H_2O(DMPC)	A	162	68	63	139	−51	58	−176	76	180	−87	63	−173
	B	170	−76	−46	−161	64	169	175	124	169	−126	63	103
sn-glycero-3-phosphorylcholine (GPC)[15]	1	−165	−71	−54	−138	73	172	−63					
	2	−172	64	65	140	−75	−63	−69					
sn-glycero-3-phosphorylcholine $CdCl_2.3H_2O$[9]		168	−69	−73	178	73	171	59					
1-lauroyl-propandiol-3-phosphorylcholine.H_2O (LPPC)[7]		162	86	45	129	84	28	78					156
1,2-dilauroyl-*rac*-glycero-3-phosphorylethanolamine.CH_3COOH (DLPE)[5,6]		−154	58	66	106	67	−52	−172	97	179	−119	65	−178
sn-glycero-3-phosphorylethanolamine.H_2O (GPE)[16]		−173	−81	−81	163	55	166	−67					

Remaining β and γ torsion angles are approximately *antiplanar* (180°).

* For notation of torsion angles see Fig. 2 legend.

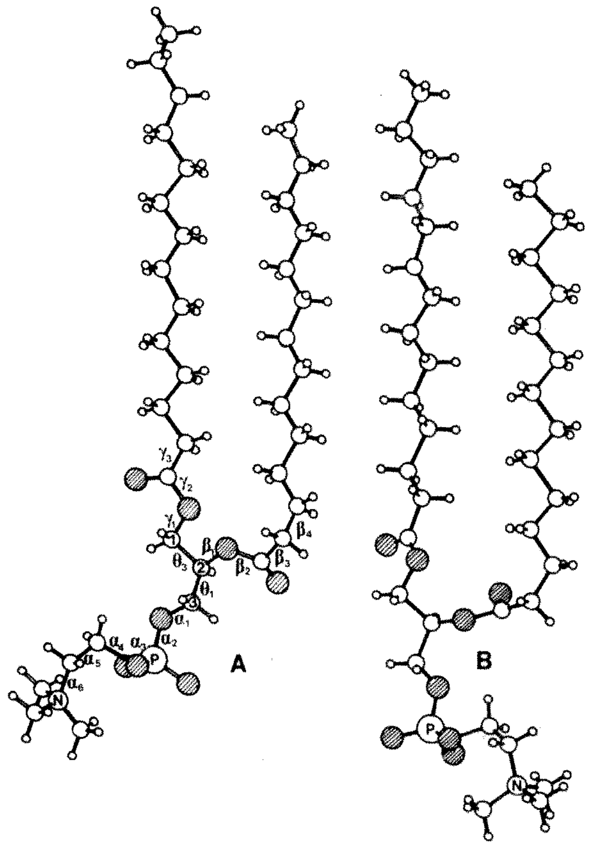

Fig. 2 Molecules A and B of dimyristoyl lecithin in a view along the unit cell *a*-axis. Hydrogen atoms are added at theoretically calculated positions to improve the perspicuity. The notation of torsion angles according to Sundaralingam[17] is indicated for molecule A. The figure illustrates the mutual 2.5-Å displacement of the two molecules in the direction of the hydrocarbon chains and the 3.7-Å offset between the two hydrocarbon chains within each molecule caused by the characteristic chain stacking.

inversion between enantiomeric conformations. The DMPC molecules A and B and also DLPE and LPPC obviously represent different energetically equally favoured stages of the freely rotating polar group.

In spite of the different orientations and mirror image conformations of the polar group, the diglyceride part is very similar in both DMPC molecules (Fig. 2). The three glycerol carbon atoms and the *sn*-1-fatty acid together form an *antiplanar* zigzag chain which is only slightly inclined (12°) from the layer normal. The initial part of *sn*-2-fatty acid extends perpendicularly (layer parallel) from this straight backbone but bends off at the second carbon atom to become parallel to the *sn*-1-chain. The same type of chain stacking is found in the DLPE crystal and, according to recent NMR studies[13], is also adopted by lecithin and phosphatidylethanolamine in hydrated bilayers and *Acholeplasma laidlawii* membranes. This diglyceride conformation must therefore be considered as a general, preferred feature in membrane phospholipid arrangements. The axial displacement of the two fatty acid chains of 3.7 Å by three methylene groups, which is caused by this chain stacking, is to some extent compensated in natural phospholipids by different chain lengths of the two fatty acids. In egg lecithin[14], for example, the predominant chain lengths in the *sn*-1 and *sn*-2 positions are 16 and 18 carbon atoms, respectively. This will reduce the interdigitation of the hydrocarbon chain ends at the bilayer centre.

In the DMPL structure the carbonyl oxygens of the fatty acid chains do not seem to take part in polar interactions, although the oxygens of the *sn*-2 chains (2 and 4 in Fig. 1) are exposed to the polar group region. These oxygens should become more easily available for interaction if the cross-section of the molecule is increased, due to either increased hydration of the polar groups and/or expansion of the chain matrix on transition to the liquid-crystalline state, which is the usual condition in natural bilayers. This accessibility of the carboxyl group of the *sn*-2-chain at the hydrophobic/hydrophilic interface of the lipid bilayer may also explain why enzymes which split fatty acids from phospholipids, such as lecithin cholesteryl acyl transferase and phospholipases of type A, have mostly evolved a specificity for this *sn*-2-fatty acid.

From available data it is obvious that the characteristic conformational features exhibited by DMPC in the crystal structure are also maintained in less ordered lecithin structures in an aqueous environment. The detailed three-dimensional structure presented here provides the necessary basis for a more conclusive interpretation of biochemical phenomena and physicochemical data recorded on model systems and natural membranes.

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Heat-shock induced proteins present in the cell nucleus of *Chironomus tentans* salivary gland

M. Vincent & R. M. Tanguay

Génétique Humaine, Centre Hospitalier de l'Université Laval, Québec, Canada

The heat shock (HS) system has been largely studied in *Drosophila* but the molecular mechanisms responsible for the induction of the heat shock genes as well as the function(s) and the intracellular localisation of the induced proteins is still unknown. It has previously been shown that the HS puff induction is accompanied by a local increase of nuclear non-histone proteins (NHP) but the nature of most of the proteins accumulating is unknown[1]. We have investigated the effects of a heat shock on *Chironomus tentans* salivary glands, a system where it is possible to study constituents in various subcellular or intranuclear regions including individual puffs[2,3], by microdissection. We report here evidence that at least two of the polypeptides synthesised in response to the heat shock migrate to the nucleus. Furthermore, these two proteins appear to have a broad intranuclear distribution, as shown by their presence in the various microdissected nuclear fractions.

At the cytoplasmic level, the heat shock (39 °C) response of *Chironomus tentans* salivary glands is similar to that observed in *Drosophila* (Fig. 1*a*, *b*). Several new proteins appear (arrows,

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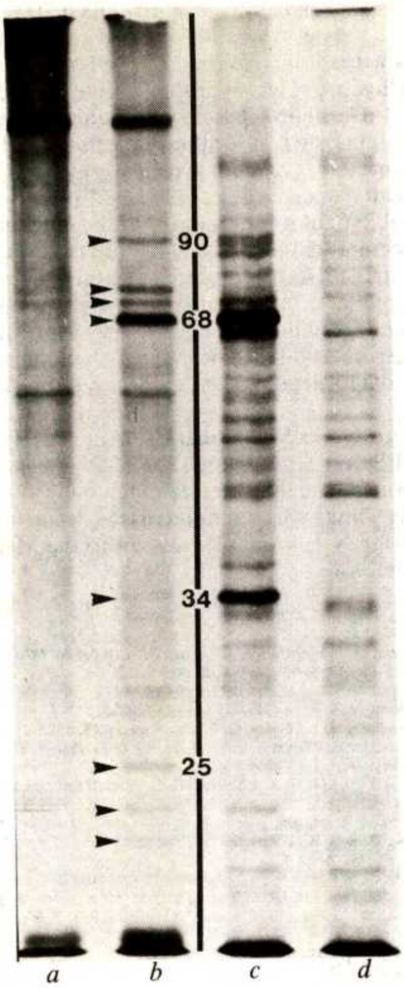

Fig. 1 Photograph of a fluorogram showing the labelled proteins from the cytoplasms of control *a* and heat-shocked larvae *b* and from the nuclei of heat-shocked *c* and control larvae *d*. Larvae were incubated at 39 °C for 10 min, and control larvae at the same developmental stage, were kept at room temperature. For both treatments, 10 salivary glands were excised and incubated for 90 min in 50 μl Cannon's modified medium (without leucine) supplemented with 100 μCi ^{3}H-leucine. After fixation of the glands in absolute ethanol:acetic acid (3:1), the glands were microdissected to obtain pure intracellular compartments. The proteins from the pooled components were extracted and analysed on a 8.75% acrylamide-SDS slab gel as described elsewhere[4,5]. The labelled polypeptide bands were revealed by fluorography[7]. In this experiment, 200,000 c.p.m. of each microdissected extract were put on the gel which had been exposed for 3 d. Arrows on *b* indicate cytoplasmic HS peptides. Approximate molecular weights, in kilodaltons are indicated in the middle of the photograph.

Fig. 1*b*) with a major one of molecular weight (MW) about 68,000. (R.M.T. and M.V., in preparation.) This protein apparently has the same MW as the major heat-shock induced peptide in *Drosophila* cell cultures and salivary gland cells. Also, normal protein synthesis is repressed in response to a 39 °C heat shock (by ~50%), as in *Drosophila*. Thus, even though the sites and numbers of heat-shock induced puffs have not been studied in *Chironomus*, it seems that its response at the protein synthesis level is quite similar to that of *Drosophila*. *In vivo* treatments (5–20 min at 39 °C) gave similar results with the exception of a gradual decline in total protein synthesis. More drastic treatments, such as a 40-min shock at 39 °C or a 10-min shock at 41 °C, resulted in an almost complete shutdown of protein synthesis (data not shown).

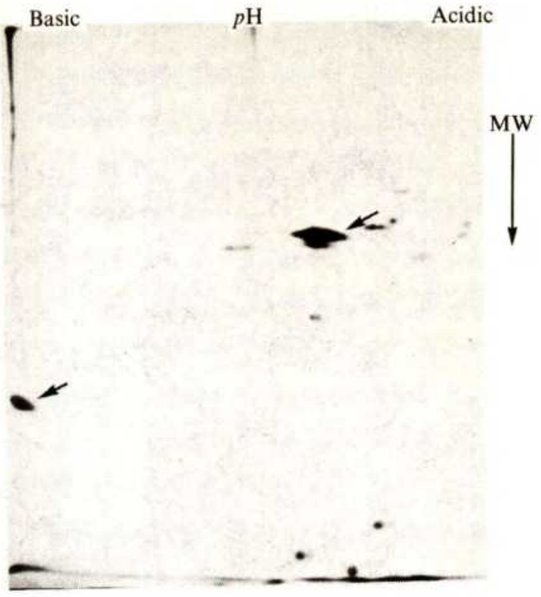

Fig. 2 Photograph of a two-dimensional fluorogram showing the labelled proteins from the nuclei of heat-shocked larvae. Isoelectric focusing in the first dimension was carried out in 9.5 M urea and SDS-electrophoresis in the second dimension was on a 8.75% acrylamide gel as described by O'Farrell[8].

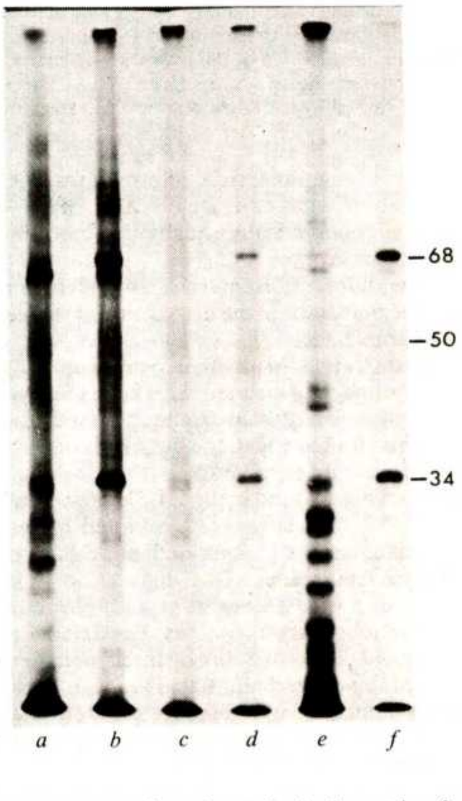

Fig. 3 Fluorogram of newly-synthesised proteins from subnuclear microdissected compartments. *a* Chromosomes from control glands; *b* chromosomes from heat shocked glands; *c* nuclear sap, control; *d* nuclear sap, heat shock; *e* nucleoli, control; *f* nucleoli, heat shock. Molecular weight markers were bovine serum albumin (68,000), γ-globulin heavy chain (50,000) and tropomyosin (34,000). Experimental procedure as described in Fig. 1 legend.

In a search for possible nuclear modifications accompanying the heat treatment, we analysed newly synthesised proteins from microdissected nuclei (Fig. 1*c*, *d*). The nuclear protein pattern from treated cells shows two major peaks, at MW 34,000 and 68,000 (Fig. 1*c*), not found in the control nuclei, the latter migrating similarly to the major cytoplasmic protein. However, the 34,000-MW protein, while being a major component of the HS nucleus, is barely visible on the HS cytoplasmic profile (Fig. 1*b*), suggesting a rapid migration of the polypeptide to the nucleus.

On the other hand, the two-dimensional pattern of the heat-induced nuclear proteins (Fig. 2) clearly established that these two peptides have distinct isoelectric points, arguing against a precursor–product relationship between them. These results provide the first indication for a nuclear role of some of the HS induced proteins.

Several minor bands on the HS cytoplasmic and nuclear profiles (Fig. 1*b*, *c*) are common to both of them. These could represent additional HS proteins which are also transferred to the nucleus. However, they could also result from minor contamination of the dissected nuclei with cytoplasmic material.

We examined the intranuclear localisation of these two proteins using microdissection techniques. The nuclei were dissected into three fractions—chromosomes, nucleoli and nuclear sap—and the proteins from each compartment were extracted and analysed by SDS-polyacrylamide gel electrophoresis as previously described[4,5]. In addition to containing the chromosomal proteins present in the control (Fig. 3*a*), the chromosome fraction from heat-shocked cells (Fig. 3*b*) shows two extra proteins with MWs of 68,000 and 34,000. These two proteins may be present in the HS-treated nuclear sap (Fig. 3*d*) simply because they are passing through it, but the possibility that they have some function there cannot be excluded. Finally, the nucleolar pattern shows some interesting features: the low molecular weight proteins characteristic of control nucleoli (Fig. 3*e*) are absent in the treated gland nucleoli, while the two HS-induced polypeptides are heavily represented in this fraction (Fig. 3*f*). In summary, these results show the migration to the nucleus of at least two newly synthesised HS-induced proteins which appear to have a general intranuclear distribution.

This provides new information about these proteins and suggests a nuclear role for some of them. There has also been an indication that HS proteins are present in *D. melanogaster* nuclei[6]. These proteins may be involved in a general mechanism responsible for the functional or morphological maintenance of the HS nucleus or in various processes such as the silencing of preexisting puffs and the transport or processing of the induced mRNAs.

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Quaternary structure-induced photoreduction of haem of haemoglobin

Teizo Kitagawa

Institute for Protein Research, Osaka University, Suita,
Osaka 565

Kiyoshi Nagai

The Second Department of Physiology, Nara Medical College,
Kashihara, Nara 634, Japan

Spectroscopic studies have provided extensive information on the primary process of visual pigments and photoexcitation of chlorophyll as well as their effects on the surrounding polypeptide chain, but a dependence of photoreactivity on the higher-order structures of protein has been observed only rarely. Resonance Raman spectroscopy can reveal the vibrational frequencies of the chromophore in a molecule provided the excitation wavelength is in the absorption band of that molecule. As the visible absorption bands of haemproteins are due to $\pi\pi^*$ transitions of the porphyrin ring, we can selectively observe the vibrational frequencies of iron porphyrin during *in situ* interactions with immediate amino acid residues of protein when the wavelength of excitation light is close to the Soret or Q band. Correlation of some vibrational frequencies of haem with the oxidation and spin states of the haem iron has been studied in detail and an empirical rule has been established[1,2]. This method is therefore especially suitable for the study of an effect of higher-order structures of protein on the chromophore. We report here a photoreaction facilitated by a particular quaternary structure of protein—in various haemoglobins resonance Raman spectroscopy showed that reversible photoreduction of haem took place in the T state but not the R state.

Haemproteins containing iron porphyrin as a prosthetic group function as oxidases, oxygenases, carriers and reservoirs of oxygen, or electron carriers in a respiratory chain in most organisms. Haemproteins do not normally serve as photoreceptors—photodissociation of axial ligands such as O_2, CO and NO bound to the Fe^{2+} ion was previously the only known photoreaction of haemproteins[3]. Methaemoglobin has been thought to be photostable, but during resonance Raman studies of the quaternary structures of haemoglobin (Hb), we found that a haem of some met-Hb in the T quaternary structure (low affinity for oxygen), but not in the R quaternary structure (high affinity for oxygen)[4], was extensively photoreduced on irradiation by laser light at 441.6 nm. The apparent photoreduction of haem was reversible and dependent on the wavelength of the irradiated light.

Figure 1 shows the resonance Raman spectra of various Hb molecules in the frequency region 1,200–1,700 cm^{-1}. It is well established that in this frequency region the resonance Raman spectra of the two oxidation states are distinctly different[1,2]. Deoxy-Hb (Fig. 1*a*) and aquomet-Hb (Fig. 1*c*) exhibited the characteristic Raman lines of their oxidation states at 1,355 and 1,371 cm^{-1}, respectively. When inositol hexaphosphate (IHP), an effector stabilising the T structure, was added to aquomet-Hb, prominent Raman lines appeared at 1,371 and 1,355 cm^{-1} (Fig. 1*b*). Furthermore, extra Raman lines were also observed in a lower frequency region (not shown) and their frequencies (366, 341, 302 and 216 cm^{-1}) coincided with those of deoxy-Hb[5]. In the frequency region above 1,500 cm^{-1}, relative intensities of Raman lines were altered by laser irradiation and the intensified Raman lines reasonably corresponded to the Raman lines of deoxy-Hb. These facts suggested that the haem of aquomet-Hb in the T structure was partially photoreduced by laser irradiation.

Carp aquomet-Hb in the presence of 2 mM IHP is known to adopt the T structure[6]: in a stationary cell this gives the spectrum shown in Fig. 2*d*. There were Raman lines at 1,355 and 1,371 cm^{-1}, as for human aquomet-Hb, implying that carp aquomet-Hb in the T structure was also partially photoreduced. When the same sample was measured in the spinning cell (1,800 r.p.m.), the spectrum (Fig. 1*e*) was indistinguishable from that observed in the absence of IHP. It was previously noted that photodissociable haemproteins were apparently protected from photodissociation in the spinning cell[7]. The actual mechanism involves recombination of the ligand during passage of the

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molecules through the dark. Thus it is probable that in the spinning cell the photoreduced Hb re-oxidised while it passed through the dark. As in the spinning condition a Hb molecule was irradiated by laser light for 0.3 ms every 30 ms, the time required for re-oxidation must be less than 30 ms. The alternative would be that the photoreduction did not take place within 0.3 ms.

Figure 2 shows the Raman spectrum of carp aquomet-Hb in the presence of IHP measured at three different power levels of laser light. The Raman line of the photoreduced species at 1,355 cm^{-1} became relatively more intense when the power level was increased. A similar phenomenon was observed by Cheung *et al.*[8] for etioporphyrinato-Ni(II) excited at 404.6 nm. They reported that tight focusing of the incident laser light at the sample point resulted in a frequency shift of a Raman line from 1,384 to 1,375 cm^{-1} and weakening of other Raman lines. The present observation differs in that two Raman lines were observed simultaneously—one at 1,371 and one at 1,355 cm^{-1}. The line at 1,371 cm did not completely disappear even in an anaerobic condition and the intensity ratio, 1,371/1,355 cm^{-1}, was unaltered by prolonged irradiation (up to 1 h). The photoreduced species was not recognised on irradiation of laser light at 488.0 and 514.5 nm. Other met-Hb derivatives including fluromet-Hb, azidomet-Hb and cyanomet-Hb were almost equally photoreduced when IHP was present at neutral *p*H. All these facts indicated that photoreduction of the haem of met-Hb apparently took place when met-Hbs were in the T quaternary form but not in the R form, and that the photoreduction was reversible. Furthermore, the lifetime of the photoreduced species was extremely short.

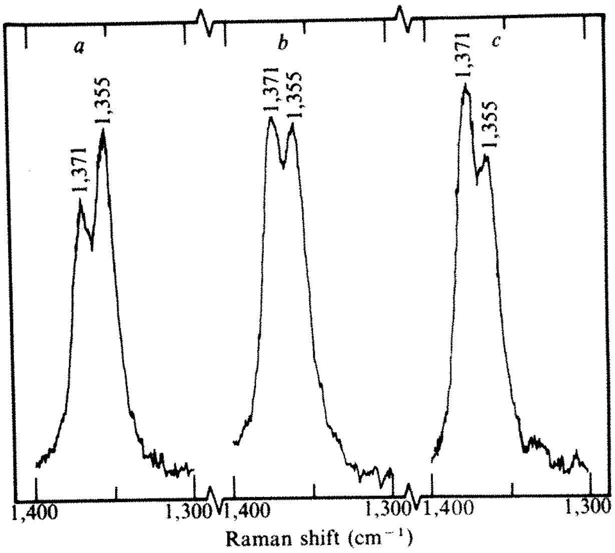

Fig. 2 Raman spectra of carp aquomet-Hb in the presence of 2 mM IHP in the frequency region 1,300–1,400 cm^{-1}. The Raman spectra were excited by the 441.6 nm line of He–Cd laser at the power level of *a*, 12 mW; *b*, 3 mW and *c*, 1.5 mW. Laser light was attenuated by the 1/4 and 1/8 ND-filters.

Photoreduction of haem, similar to that reported here, was recently observed for cytochrome oxidase on irradiation of laser light near the Soret band[9–11]; the electron donor was thought to be a flavin impurity. In the present case, however, the Hb solutions used for the T and R forms were identical except for the presence or absence of IHP. Therefore the electron donor for photoreduction is endogenous and may possibly be axial ligands of haem iron. Whatever the electron donor involved, this study suggested that an electron was reversibly transferred to haem from the donor only in the T structure while the oxidation state of the whole molecule remained unaltered, and that the rate of electron transfer depended on the wavelength of irradiated light, presumably as a result of the wavelength-dependent electronic excitation of the acceptor group.

We thank Kinmon Electric Co. for the use of a He-Cd laser, and Mr T. Ogawa for providing carp.

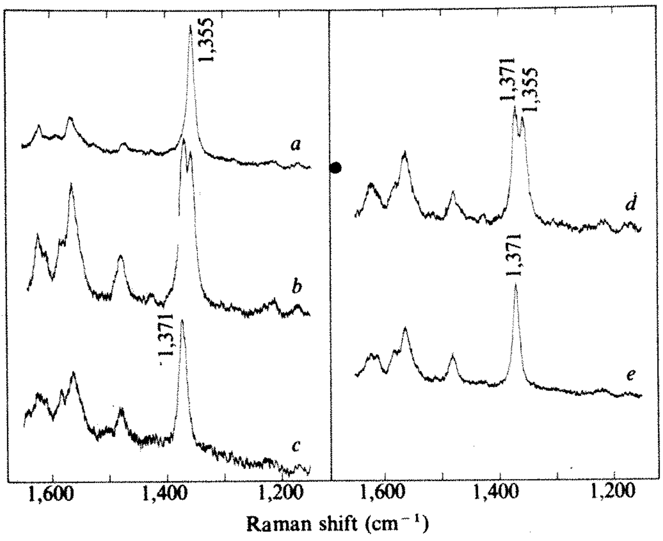

Fig. 1 Resonance Raman spectra of human and carp aquomet-Hbs in 0.05 M bis-Tris, 0.05 M Tris buffer *p*H 6.5 excited at 441.6 nm (He–Cd laser, Kinmon Electrics). *a*, Human deoxy-Hb A; *b*, human aquomet-Hb A in the presence of 2 mM IHP; *c*, human aquomet-Hb A in the absence of IHP; *d*, carp aquomet-Hb in the presence of 2 mM IHP measured in a stationary cell; *e*, the same sample as (*d*) but in a spinning cell (1,800 r.p.m.). Human and carp haemoglobin were prepared as described by Kilmartin and Rossi-Bernardi[12] and Tan *et al.*[13], respectively. Hb was oxidised at *p*H 6.9 by addition of 1.2 equivalence of $K_3Fe(CN)_6$ and then was stripped of phosphate by passage through a Dintzis column.

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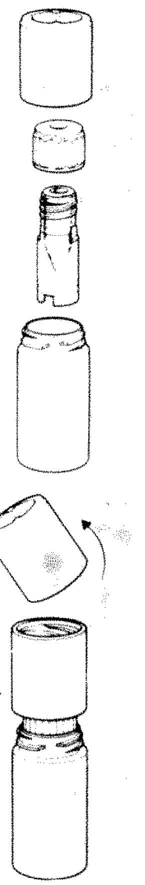

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reviews

Members of the club

Thomas H. Jukes

The Eighth Day of Creation: The Makers of the Revolution in Biology. By H. F. Judson. Pp. 686. (Simon and Schuster: New York; Cape: London, UK, 1979.) $15.95; £7.95.

George Gamow, about 1954

THE book is in three sections. The first deals, in much detail, with events leading to the discovery of the structure of the DNA molecule. It is a comparatively straightforward topic. The third section is about the structure and function of protein molecules, and the procedures, especially crystallography, that were used in research with proteins. This subject, quite complex for the average reader, lends itself to logical treatment, and the author attacks it with obvious relish. He was helped by his friendly relationship with Max Perutz.

The intermediate section is about molecular biology from the time of the discovery of the DNA formula to the solution of the genetic code. This is a difficult amd controversial era for evaluation because of the involvement of many scientific groups, and this is therefore the most personalised and subjective part of the book.

The challenge to genetics and evolution, posed by Watson and Crick in 1953, was a biochemical one: to find out how information travelled from the gene to the phenotype. As described by Judson, enter George Gamow, an ebullient and articulate cosmologist. There followed a strange period in which the mysteries of molecular biology were planned to be solved by a special coterie called the "Tie Club", the membership of which was limited to 24, one for each nucleotide in DNA and each amino acid. Crick was the scoutmaster of the troop, and merit badges (ties and tie clasps) were awarded and issued by Gamow. Apparently, this cabal took itself seriously, and exchanged "Tie Club" letters, on scientific concepts and developments.

All who have followed molecular biology have impressions and memories of the years (1953–1965) between the announcement of the DNA helix and the completion of the genetic code. I suppose that leadership in science is based on a blend of performance and eloquence. Certainly the scientific stars who dominate Judson's narrative were great talkers, with dominant personalities. Francis Crick can hold a packed auditorium spell-bound by a loop of string and a description of supercoiling and writhing. No wonder Judson was swept away, and he tends to be reverent in the presence of the great. For example (page 92):

> "We now have standardized people with respect to their intake by giving them a standardized small-molecule diet" — he gestured towards the packet of Vivonex 100 — "which is immediately absorbed from the stomach into the blood stream, no digestion, the contents of the gut quickly disappear and the bacteria too. They are starved to death on this diet. Perfectly nutritious . . . ."

This prestidigitation by Pauling was swallowed by Judson, together with the Vivonex, and 2g of vitamin C. He says: "I'd been captured by that infectious grin and that unquenchable self-confidence . . . .". Self-confidence does not change the stomach wall, which, in the absorption of food, "behaves as if it were made of porcelain". If not, the liver would be promptly marinated by hydrochloric acid secreted in the gastric juice. Bacteria do *not* disappear from the gut when food is withheld.

A dinner at King's College (1971) inspires Judson to unctuous prose (p221):

> From my right, along the polished wood, there arrived a little trolley train on wheels, in the shape of a silver swan, with three parts joined by swivels, and each part bearing a decanter. A card said that the port was Taylor 1955, the white was Raventhaler Herberg Riesling Spatlese Cabinet 1959, and the red was Corton Les Maréchaudes 1962. Beyond Brenner sat a gray-haired woman, a computer mathematician from one of the women's colleges. Brenner leaned towards her. "There will be no difficulty in computers' being adapted to biology", he said, with clenched teeth. "There will be Luddites. But they will be buried".

Perhaps Sydney was getting a bit edgy about public objections to genetic manipulation.

Dounce's paper on coding (*Enzymologia,* **15**, 251; 1952) is given courteous but somewhat brief treatment on pages 247–248. His proposal was that groups of three consecutive bases in RNA each furnished the code for an amino acid; and Yčas (in *The Biological Code;* North-Holland: Amsterdam and London, 1969) states that Dounce " . . . . also suggested that the information in the RNA template may be stored in DNA. 'If . . . . genes are composed of deoxyribonucleic acid, then the deoxyribonucleic acid gene molecules would act as templates for ribonucleic acid synthesis, and . . . . the riboncucleic acid synthesized on the gene templates would then in turn become templates for protein synthesis in the nucleus or cytoplasm or both'. Dounce's proposal thus clearly stated what became known later as the 'sequence hypothesis', as well as the messsenger RNA concept.".

Even though Dounce's proposal preceded 1953, Judson devotes excessive space to free-swinging and palpably erroneous speculations on coding by Gamow. It must have been a blow that Nirenberg and Matthaei were not "members of the club" (p447), for according to Meselson, results by non-members were "unlikely to be correct" (p481). However, recovery from the shock was rapid.

Of course, it was the announcement by Nirenberg and Matthaei, and its consequences, that finally brought genetics within the grasp of biochemistry. Judson's criticisms (p487) that the experiments with random polynucleotides failed "to allow for synonyms" are not well taken. The degeneracy of the code was shown by the "shared doublets" and most of the coding assignments were correct; except that the sequence of bases in each triplet was not discernible until later. An epilogue soon followed, although Judson does not mention it: Fred Sanger got tired of waiting for his turn to use the amino acid analyser, so he decided to sequence nucleic acid molecules.. (He had previously shown everyone how to sequence proteins.) Place the amino acid sequence of a virus coat protein beside the sequence of the viral RNA molecule, and there you have the complete story — the direction of reading and all the codons. But, apparently, Fred spends too much time in the lab, and not enough in talking to historians.

Judson gives the genetic code the most perfunctory treatment of any major topic discussed, except for long summaries of early theoretical work that turned out to be in vain. The first preliminary indication of code composition was by Sueoka (1961) who showed that high A + T in DNA of bacteria was correlated with isoleucine, lysine, phenylalanine and tyrosine; high G + C with alanine, arginine and glycine in bacterial proteins.

Crick showed great insight in insisting that exactly 20 amino acids were used in protein synthesis (pp252-258). For years afterwards, biochemical authors still frequently talked of "about 20", or "about 22". Hydroxyproline in gelatin was particularly confusing. Asparagine and glutamine were often omitted although isotopic experiments had shown they were incorporated intact. Judson errs in stating that Gowland Hopkins, in 1901 "had been first, with tryptophan, to isolate an amino acid". Glycine was isolated from gelatin hydrolysate in 1820; tryptophan was seventeenth.

L to R: Jacob, Delbruck, Meselson, Rolfe, Stent and Brenner

Arthur Pardee, 1960

The story of the messenger concept is developed in detail. I well remember the excitement caused by the "DNA-like RNA" finding by Volkin and Astrachan in 1956. The statement that this work "could not quite be dismissed as the inconsequential and self-admittedly sloppy work of people not well known at a lab not highly regarded" (p325), is an unjustified slur on the lab where Cohn and Volkin (*Nature,* **167,** 483; 1951) pioneered on phosphate linkages in nucleic acids. Also in 1956, Fraenkel-Conrat, and Schramm, showed that tobacco mosaic virus RNA was by itself infective and carried all the information needed to make the virus. In 1960 (pp432-446) various efforts succeeded in establishing the existence of messenger RNA. However, in 1961, messenger RNA was upstaged by polyuridylic acid, and we learn (pp475-479) that Nirenberg and Matthaei had never even heard of messenger RNA!

Judson, to some extent, follows Watson's style be describing personal characteristics of superstars. The less ostentatious scientists come off second best. Judson says (p581): "what Pardee lacked, perhaps, was the knack of generalization". For "generalization", read "self-glorification". Stent, who is a sort of Louella Parsons of molecular biology, is quoted (p405) as saying that Pardee is its "Douanier Rousseau", an analogy that I found strange. Pardee is probably more of an orginator (like Chagall?) than a primitive. Judson's preoccupation with the personal life of his luminaries sometimes becomes excessively chatty. For example (p283):

> "On 6 December 1954, Stent wrote to Brenner, saying, in part:
>
> Dearest Sydneyboy!
>
> The Lwoffs have just left, and I have a few moments between cocktail-parties to dash off a couple of lines, so that there'll be news from us awaiting you when you make your triumphant return to the Rand. Ta ever so for your Oxonian and Cantabrian letters which both Inga and your serviteur devoured with the greatest of that old gusto. Boy do we miss you here! You simply *must* come back to Berkeley. In future letters, I hope we can discuss in more detail just what we can do to bring you and your gang for an extended stay back to Bagdad-by-the-Bay . . . .
>
> So, Wolfgang Amadeus von und zu Witwatersrand, receive thousands of amitiés from your ever-faithful
>
> Inga and Gunther"

But Judson does well with prolonged and detailed scientific descriptions in a variety of fields, especially crystallography and protein structure. His book will be widely read by scientists; and of course, the handful who are apotheosised by it will love it, as will their admirers.

Throughout the book, one becomes aware of a race between two schools — on one hand, deductions made by molecular geneticists and theorists, and on the other, the staunch empiricism of biochemists. The fine structure of the gene was solved by Ingram's demonstration in 1956 that glutamic acid at the sixth residue of ß haemoglobin was replaceable by mutation to either valine or lysine. Benzer's approach to this was by genetic analysis of rII mutants in bacteriophage T4, which reached some kind of a peak in a six-page fold-out that was inserted in *Proc. natn Acad.Sci. U.S.A.* in 1961. The inexorable progress of biochemistry brought the tangible emergence of transfer RNA, soon following its prediction by Crick's brilliant adaptor hypothesis. On p341, an account of the genesis of the RNA cloverleaf structure would have been welcome. The formula for glucose on p378 is incorrect.

In this book, some of the prominent scientists whose personalities were familiarised by Jim Watson, continue to cavort as they did in *The Double Helix*, and are joined by other performers. We are spared few details, even unto the length of the hairs in Brenner's eyebrows (up to three inches). But Judson's careful and sympathetic account of the life of Monod, developed at great length, is good reading.

Will *The Eighth Day of Creation* be studied as history, read as a novel, or used as a textbook? My own feeling is that the history of molecular biology deserves, for documentary purposes, a more dispassionate and even-handed treatment by someone who will undertake a task comparable in detail to that of Judson.

Thomas H. Jukes is in the Department of Biophysics and Medical Physics, University of California, Berkeley, California.

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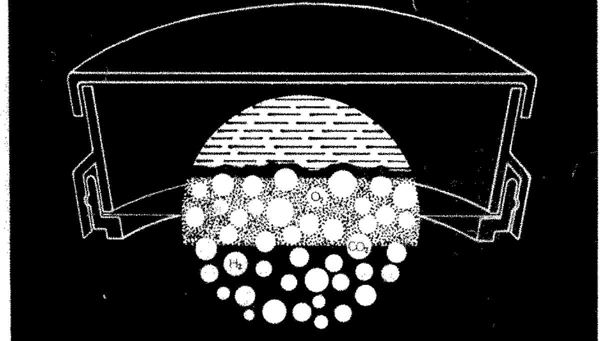

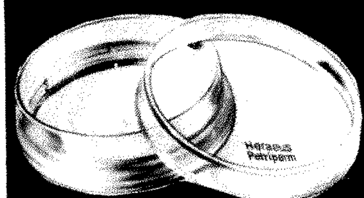

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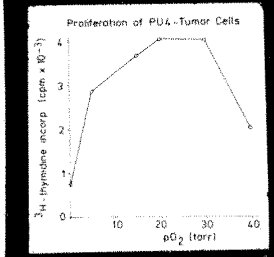

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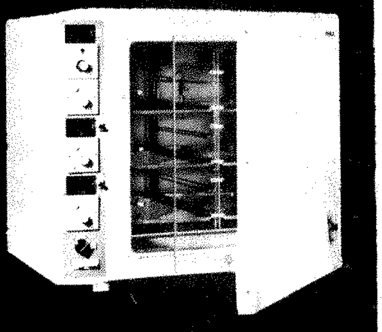

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Switching of globin genes

Cellular and Molecular Regulation of Hemoglobin Switching. Edited by G. Stamatoyannopoulos and A.W. Nienhuis. Pp. 792. (Grune and Stratton: New York, San Francisco and London, 1979.) $68.50.

THIS multi-authored book contains the proceedings of a conference held in June 1978 which aimed to review the areas of haemoglobin synthesis and red blood cell differentiation pertinent to developmental and physiological 'switching' of globin genes. The sheer volume of information in this book is staggering, and it is difficult in this review to do justice to all 48 articles. The style of presentation was left to individual authors and I found most papers very readable, with many contributors presenting their work in a more general, and generous, way than is usually found in the literature. One disappointing feature of the book is that it lacks a general overview of the type previously published for the meeting (see *Cell,* **15**, 307-315; 1978), and this leaves the reader to judge how far the field had progressed in 1978.

The book is divided into three main sections covering the changes in haemoglobin synthesis during development, haemoglobin switching and erythroid differentiation, and the molecular biology of globin genes. The first article by Weatherall clearly describes what is known about the genetics of human disorders that are characterised by the persistent synthesis of fetal haemoglobins after birth, and subsequent papers examine human fetal globin chain synthesis *in vivo.* Using animal models for studying the 'switching' of haemoglobins, the concept of stress erythropoiesis in baboons (De Simone *et al.*) and in sheep (Wood *et al.*) is introduced, leading to a series of articles describing the controls of haemoglobin synthesis in these model systems.

Attention is next focused on erythroid precursor cells with articles showing the hormone influence on differentiation and the differences between stem cells isolated from bone marrow and peripheral blood. Highlights of this section include a review by Eaves on erythropoietic differentiation. Rapid progress has been made in the culture of haemoglobin-producing cells *in vitro* and Nienhuis shows examples of this in an article on 'switching' in cultured sheep stem cells. Ingram proposes a provocative model to explain the possible effect of various chemical inducers on the metabolic events in Friend cells which leads to differentiation and the subsequent accumulation of globin mRNA species.

The final section of the book deals exclusively with the molecular layout of globin genes and includes an elegant description of mouse β-globin gene arrangement by Leder and an explicit guide to cloning genomic DNA in λ bacteriophage by Lacey *et al.* A series of three papers show how the human globin gene map looked in 1978. Globin mRNA is well reviewed in a series of articles covering such topics as sequence, modification, processing, and developmental changes. In the final part entitled "Control of globin gene expression", Weintraub proposes how classes of nuclear protein may interact with DNA in active genes conferring the conformation required for specific expression; this is the only paper to mention nucleosomes. Young *et al.* show the use of DNase I for probing active globin genes in fetal and anaemic sheep, their results indicating quite different mechanisms for gene activation and deactivation. Finally Deisseroth and Hendrick explain their results with cell fusion and Anderson *et al.* report the progress in trying to use cell microinjection as a potential assay for those elusive regulatory factors.

I feel sure researchers in the haemoglobin field will find this a valuable reference book. It also offers much to the clinician attempting to understand the newer areas of biology.

Graham Cowling

Graham Cowling is in the Department of Biophysics, King's College, University of London, UK.

Rotating stars

Theory of Rotating Stars. By J.-L. Tassoul. Pp. 506. (Princeton University Press: Princeton, New Jersey, and Guildford, UK, 1979.) Hardback £25; paperback £9.40.

ROTATION pervades the domains of astronomy at every level: planetary, stellar, galactic, and beyond. This book is devoted to the stellar level. The study, both observational and theoretical, of rotating stars is a growing speciality among astrophysicists. No other reference work in the subject even attempts the breadth of coverage presented in this book. It will be heavily used, not only by specialists, but also by non-specialists looking for a concise and authoritative picture of the status of one or another branch of the subject. The explantions are sufficiently clear and self-contained that it could serve as a graduate-level text.

The first two chapters summarise the historical development and observational background, emphasising the problems confronting theory, thereby motivating the choices of theoretical questions found in the remainder of the book. These are followed by a chapter on hydrodynamics, specialised to the conditions prevailing in stars. This is an especially important chapter. It underlies the rest of the book in the sense that virtually all questions subsequently addressed are formulated as hydrodynamical problems. It is not an easy matter to develop hydrodynamics in one chapter (in fact, certain special aspects of the subject, stability theory in particular, are developed in chapter six). Tassoul does a very good job in my opinion, both in explaining the subject matter and in guiding the reader to the literature. For example, specialists in stellar hydrodynamics have had an unfortunate tendency to ignore the large and related body of material in geophysical hydrodynamics. Tassoul is careful to make the appropriate references to the geophysical literature.

Chapters four to seven are very much preparatory, theoretical, and hydrodynamical in nature, covering steady-state models of rotating axisymmetric stars, techniques for constructing them, techniques for studying their stability, and some results of a general character regarding their stability. Chapter four, on permanent rotations, collects a number of classical results on the nature of the allowable rotating configurations, many of which appear only in older, relatively inaccessible texts or articles. Some are given in detail, like Hamy's proof that a centrally condensed barotrope cannot be stratified on concentric spheroids. Others are only stated, like Lichtenstein's theorem that a uniformly rotating and uniformly dense self-gravitating mass possesses an equatorial plane of symmetry. In chapter five, a number of approximate methods, perturbation and numerical, for constructing rotating configurations, are described. Chapter six is devoted to an up-to-date description of stability techniques, some of which find applications in chapter seven to obtain limitations on the allowable distributions of angular momentum.

The discussion of specific subspecialties begins with chapter eight (on meridional circulation) and concludes with chapter sixteen (on rotation in close binaries). In between there are chapters on the Sun's differential rotation, on the distinctions between uniformly and differentially rotating models, on the questions of collapse and fission, on a closer examination of the relation of the models to observations, on white-dwarf stars, on oscillation and stability properties, and on magnetic fields in rotating stars.

There exist some books and review articles covering some of these narrow areas in depth, and others covering broader areas superficially. Until the appearance of this book, there existed no reference attempting broad coverage in depth. Tassoul has filled this gap with a high standard of scholarship. In some respects, his book has the appearance of a series of review articles on a variety of subspecialties, but it is much more. It is an account of the field, uniform in style and scholarship, and further unified by a particular viewpoint. The viewpoint is definitely from the theoretical side, and observational astronomers may find it uncongenial, although Tassoul has made consistent efforts to relate theory to observation.

The literature review is impressive. It must have required reading and digesting an enormous number of articles covering widely differing scientific viewpoints. The conclusions of these articles are not simply reported, but are compared, criticised, and, in some cases, synthesised. For example, in chapter eight on meridional circulation, a section of about ten pages is devoted to a critical review of the assumptions underlying the subject. Five principal assumptions are isolated and collected at the beginning of the section, and then examined one by one. This degree of thoroughness does not make light reading, but is certainly useful to anyone wanting to understand the subject in depth. Other examples of this kind of critical examination of assumptions, less formally organised, are found throughout the book.

Another, closely related, function of the book is to summarise the work of others. This is especially helpful when competing theories are summarised side by side. This is done, for example, in the cases of theories of the Sun's differential rotation (chapter 9), of the collapse and fission of protostars (chapter 11), and of the β-cepheid variables (chapter 14). It is inevitable in summarising conclusions from many subspecialties that some erroneous assertions must appear. But in reading with particular care the summaries of those areas I think I understand, I am principally impressed by their accuracy. The errors do not seem damaging. They are insignificant in comparison with the assistance the summaries provide to the otherwise unguided reader.

Nobody is perfect. Tassoul omits some topics that are currently exciting a lot of interest. The scope of a book like this does have to be limited in some way, and Tassoul's stated principle for doing so is to consider only topics of long standing from Newtonian (as opposed to general relativistic) mechanics. He does not discuss the widely accepted picture of pulsars as rotating neutron stars (because the subject is not of sufficiently long standing?) or X-ray binaries and black holes. He has apparently decided to direct attention only to those problems that have settled down to the point that their formulations (if not their solutions) are clear and not likely to change before the book gets into print. One can sympathise with this decision while recognising that it makes the book duller than it would otherwise be. It would not have required a deep excursion into the theory of general relativity to present some of the current work and at least provide references. There is a precedent for this in the book, in the chapter on close binaries: the more questionable points are not discussed in detail, but references are given.

And there are some errors. I noted a succession of minor errors in chapter 6 on oscillations and stability. For example, the definition of stability given on page 116 fails to apply to conservative systems, as it requires departures from the unperturbed state to die away with time, rather than merely remain small. Next, the commutivity of the operators of Lagrangian pertubation and of material derivative is presented only in the linear approximation (page 119), whereas it holds quite generally. Perhaps this omission is excusable, as this is the presentation given in the usual reference, but it has the effect of propagating an erroneous impression. On page 122 it is asserted that the free boundary of a fluid mass is defined by the condition that the pressure vanish there. It is possible to get away with such a definition without making mistakes, at least in a number of special cases, but it is not the right mathematical description of the physical situation. The free boundary is defined as the mathematical (or topological) boundary of the set where the density is positive, and the vanishing of the pressure there is a physical boundary condition, not a definition.

This frequency of errors is not typical. I noted a few more through the book, of the same degree of seriousness as those above: annoying, but not paralysing. Doubtless there are others I missed, but my net impression remains that the number of inaccuracies is remarkably small considering the scope of the book, and their negative contribution is negligible in comparison with the overall, positive contribution.

References are collected at the end of each chapter, related to sections within that chapter, and partially annotated, thus giving some guidance to the reader. There is an index of names, apparently quite complete, and an index of subjects, which I found incomplete.

This book should, and I feel sure will, become the standard reference for the subject matter covered. Anyone involved in the subject of rotating stars will simplify his life by having a copy on his shelf.

Norman R. Lebovitz

Norman R. Lebovitz is Professor of Applied Mathematics at the University of Chicago, Illinois.

Chinese chemistry and chemical engineering

Chemistry and Chemical Engineering in the People's Republic of China. Edited by John D. Baldeschwieler. Pp, 266. (American Chemical Society: Washington, DC, 1979.) Hardback $15; paperback $9.50.

THIS book is the report of a US delegation of chemists and chemical engineers which travelled widely in central and northeastern regions of the People's Republic of China during May–June, 1978. It is perhaps the most informative survey of chemical activities yet available. Chinese science, in modern form, began in the first half of this century and although at a rather preliminary level, seemed to be progressing slowly before 1965. In the following ten years, the influence of the 'gang-of-four' and its Cultural Revolution was serious, halting progress and destroying morale. Premier Chou-En-lai had talked in 1975 of the four modernisations, including science and technology, but only after the removal of the gang-of-four in 1976 could his principles be applied. In 1977–78 plans for re-orientations were announced by Chairman Hua Kuo-feng, and later by Vice-Premier Teng Hsiao p'ing, and Fang Yi, head of the Commission on Science and Technology, then gave full details of a seven-year programme aided at achieving parity with other big nations by the turn of the century.

There are three main sectors, the Chinese Academy of Sciences (CAS), the universities (under the Ministry of Education), and the Ministry of Production, all being coordinated and controlled by the Commission for Science and Technology (CST), whose head Fang Yi is a Vice-Premier, a Vice-President of the CAS, and a member of the Political Bureau. A few centres are run in whole or part by the provincial Government, and special divisions of the CST are dealing with energy, communications and industrial technology.

The CAS in Peking was greatly weakened in the Cultural Revolution, but its status has now been restored, and there are already some 90 research institutes across the country, covering the main fields of science, and branches of the CAS in Shanghai, Czechuan, Sinkiang and elsewhere. There are, at present, 14 Institutes for different aspects of chemistry (300–1000 staff) and scientists themselves are again playing a greater part in formulating policy, rather than the Revolutionary Committees, many old directors having been re-instated. The Chinese Academy of Medical Sciences (CAMS) has 13 Institutes in Peking and Shanghai, and links with hospitals and with the pharmaceutical industry. Research training courses for university graduates now operate in some of the Institutes.

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The universities, too, were very disturbed by the gang-of-four. Foremost for chemistry, perhaps, are Peking, Shanghai (Futan), Kirin and Canton, and for engineering Peking (Tsinghua) and Shanghai. Student numbers have increased considerably in the past two years. Entrance examinations have been re-introduced, giving first priority to intellectual ability, and there is to be far less time for political instruction. There is still a serious shortage of textbooks. The student/staff ratio is very low (average about 2.7), very different from that in European or American universities. Chemistry courses have been lengthened to four years, in which some mathematics, physics and applied science is included. Equipment for teaching is fairly good. Unfortunately, the effects of the Cultural Revolution were to create a sort of vacuum in the trained teaching staff, for the older members who had received training in Europe or America are now old or retired, and the middle-aged and younger groups were sent out to the Communes and have become dispersed. In spite of the intrinsic ability of the Chinese people, this shortage of adequate staff may not be corrected for many years. A few universities are trying to start advanced research training, but no degrees are to be given yet. Contractual applied research or actual production previously carried out in some universities has almost entirely ceased.

The pattern of industrial technology and research is complex, and under a combination of central and provincial control, as in the petroleum and chemical industries, and in the extensive research on catalysts. Scientific staff for the Taching oil development are mostly trained at the Peking Petroleum Institute, and the Harbin Petroleum Research Institute is still largely controlled by the provincial Government. Chinese students, after training, are prepared to be sent wherever their country needs them.

As might be expected, much of the current science and technology in China is directed towards practical needs. Impressive work is in progress on pharmaceuticals, especially those extracted from indigenous plants, traditionally used as medicinals; and on the synthesis of natural products and nucleic acids. These include peptides, hormones, prostaglandins, antibiotics and some anti-cancer and anti-bronchitis reagents. Much of this work is done in the Institutes of Organic Chemistry, Biochemistry and Materia Medica in Shanghai, and those of Materia Medica and Chemistry in Peking. The synthesis of insulin in the Institute of Biochemistry, Shanghai, some years ago was a famous achievement. Production of polymers, mostly the best known classes, has made good progress, with extensive research on improved catalysts for polymerisation. The Peking Institute of Chemistry has a large division on polymer research. There and in other Institutes, new fluoropolymers, crown-ether type polymers, and others with functional groups having pharmacological or other useful properities, are being studied.

Excellent work is being done in the Institute for Ceramics and Metallurgy, Shanghai, including the growth of single crystals, ferrites, ceramics for the electronic industry and for high temperature equipment, diamonds, and crystals with special optical and electrical properties. Research in the petroleum and petrochemical fields is concentrated in Institutes at or near refineries or industrial complexes in some of the major cities. There is conventional work on the evaluation of crude oils, catalytic cracking and reforming of fuels, catalysts, lubricating oils and additives, engine testing and adhesives. Close contact between the Institutes and production plants is maintained. The manufacture of petro-protein using yeast is being explored, the products being tested as animal feedstuffs.

Rare earth ores are abundant in China, the elements being separated by ion exchange or through complexing reagents. Their possible use for the coating of fluorescence tubes, as converters of X-rays into visible radiation in medical equipment, and for catalysts is being explored.

By contrast, there is relatively little work on organometallic chemistry, molecular spectroscopy in relation to structure, quantum and theoretical chemistry, or reaction mechanisms and kinetics. Isotopes such as $^{18}0$, ^{15}N, ^{13}C are being produced for labelling and tracer work, high-energy physics has not yet begun. T present computer facilities are limited. With a few exceptions, there is still shortage of newer physical instruments and techniques for structural determination and analysis, such as those for nuclear magnetic resonance, X-ray crystallography, mass spectgromatry, electron or Mossbauer spectroscopy. There is much admirable home-made equipment of a less sophisticated kind, such as chromatographs. Work with lasers is still at an early stage, and studies with molecular beams have not yet started. All this is consistent with the policy of dealing with the more urgently practical applicatrions first, but this situation may change quickly.

This book gives details of all places visited, names of scientific workers, charts of the organisation of science and technology, and the state of scientific information services in China. As regards both factual comments and the opinions expressed, it agrees closely with reports of Fellows of the Royal Society who have visited China in recent years. It is an admirable account for all interested in Chinese chemistry and chemical engineering. **H.W. Thompson**

Sir Harold Thompson was formerly Professor of Chemistry at the University of Oxford and Foreign Secretary of the Royal Society. He has visited China three times: in 1962 and 1974 with delegations from the Royal Society, and earlier this year on behalf of the Great Britain-China Centre, of whose Executive Committee he is Chairman.

Trying to comprehend

The Nature of the Physical Universe: The 1976 Nobel Conference. Edited by D. Huff and O. Prewett. (Wiley-Interscience: New York and Chichester, UK, 1979). £12.75.

THIS volume collects together six lectures given at the twelth conference supported by the Nobel Foundation at Gustavus Adolphus College, Minnesota. They are concerned with our understanding of the nature of the physical Universe, and with man's place in it. The level is intended to benefit an audience of intelligent laymen.

The first four lectures, given by scientists, are breathtaking in their scope. Viki Weisskopf begins at the microscopic level with the question "What is an Elementary Particle?". He quickly traces the history of man's exploration of matter, describing the energy level systems appropriate for atoms, for nuclei, and for nucleons. With such differing depths of exploration before us, a firm reply to this question is obviously difficult, but his final answer is in terms of "*conditional elementarity*", the idea being that we can speak of elementary particles or not, only when we specify the energy resolution of our observations. The question whether quarks themselves may have structure is thus left open, for future research to settle. It is indeed a most pertinent question, now that we recognise 15 varieties of quark (5 flavours, each with 3 'colours'), since the discovery of the upsilon particle in 1978. At present, we have no evidence for any structure, or finite size, for these quarks, but this situation may change when we can experiment at much higher energies and momentum transfers.

This story is then taken on by Murray Gell-Mann, who speaks about the new quantum number of 'colour', and about the gauge theory which has been built on it, known today as quantum chromodynamics (QCD). But there are also the 'leptons', the electron and its heavier counterparts, the μ and τ leptons, and the three neutrinos corresponding to them. Gell-Mann describes the programme of unification, recently successful in uniting electromagnetic and weak interactions to form the "electro-weak interaction"; with its next goal the ronic forces with them, his grand design has been our most cherished dream, seem to be rather close to it". ion would change suddenly, if were found to have substructure.

ve Weinberg asks "Is Nature mple?". His emphasis is on symmetries, not only the symmetries of space-time known from our daily life, but also the so-called internal symmetries, associated with charge, flavours, colour, and so on. For these latter, he introduces the notion of gauge symmetries, which imply the existence of gauge fields, with corresponding quanta; these gauge symmetries then govern the interaction of the gauge quanta with quarks and leptons. In this sense it is the gauge symmetry of colour which requires the existence of a gluon field, the gauge field for colour, which carries colour itself.

Most of the internal symmetries which we have known in elementary particle physics have turned out to be only approximately true, and, as a consequence of gauge theories, Weinberg now believes that "all these approximate symmetries can be, and in fact are being, understood as dynamical accidents". His remark is made specifically for flavour symmetries and it is a remarkable conclusion today, when one recalls what a great step forward the SU(3) unitary symmetry seemed to be in the mid-1960s. This has been a remarkable change in our point of view, which has dawned on most of us only quite recently, long after Weinberg's lecture. His remark applies even to parity conservation, allowing the possibility that particles with left-hand spin may have physical properties different from their right-hand counterparts. Weinberg concludes that "it is probably too early to try to think of drawing any philosophical lesson from discoveries in physics".

Fred Hoyle's lecture is concerned with the development of the Universe, right from the first moments of the 'Big Bang'. His emphasis is on the abundances of elements today, and on the conditions which must have existed to give rise to them. He draws our attention to the observation of simple organic molecules embedded in grains of meteoric dust, seeing in them a possible interstellar origin for life, arising even before our own Solar System formed. He traces out the nature of our chemical environment and leads us to the question "What does this tell us about the long-term future of our species?" These constraints imply a number of unpleasant conclusions concerning our very immediate future, for we cannot expect "to enjoy the great luxury of living in a hydrocarbon era" for many more generations. However, if population limitation were to come soon, and we could reach zero population growth, then "nothing concerning the future of our species would look unfavourable".

The last two lectures are rather philosophical in style. Stanley Jaki of Seton Hall University lectured under the heading "The Chaos of Scientific Cosmology". His remarks about its history interested the reviewer, as we scientists hear rather little concerning older theories about the Universe, refuted by later evidence. It is odd to hear the views once accepted as reasonable by eminent scientists whose names have become law in the areas of their own scientific success. However, it is not clear whether the chaos Professor Jaki is speaking of is the chaos of this history of cosmologies, or whether it is the chaos of matter within the Universe. In his view, "the primeval chaos of fundamental particles" is connected somehow with its unreliable mathematical underpinning, unreliable because Gödel's incompleteness theorem removes any hope of demonstrating the consistency of any such mathematical system within itself. So he advises caution concerning any beliefs about scientific cosmologies or any implications they may suggest for Mankind.

The final lecture was given by Hilary Putnam, who discussed three areas lying outside present-day science: the domains of objective values, of freedom of choice, and of rationality. He rejects the notion that ethics are the result of instinct or conditioning. He considers whether our impression of free will results only from Chance but concludes that we have so little understanding of probability that this line of thought can offer no hope for any enlightment. His opinion is that the selection pressures of evolution cannot account in full for the development of ethics and rationality, an understandable view with which we will all have much sympathy. On the other hand, our ability to develop Science, and to comprehend and control our Universe by discovering physical laws and understanding how to use them for prediction, could well have arisen as a by-product of natural selection, in the reviewer's opinion. The situation does not call for the existence of any selection pressures directed specifically towards scientific activity. Also, our development of Science is not the result of testing random hypotheses, nor is it the end result in a chain of mathematical logic, the possibilities considered by Professor Putnam; it lies somewhere between, guided by the mind's ability to react to needs, to see analogies, and so to relate things apparently rather different, all characteristics which may simply be by-products of the survival value of rationality.

To this reviewer, the contrast between the earlier and the later lectures in this series illustrates well the enormous gulf between scientists and philosophers today, a gulf which is probably as large as it has ever been. Scientists have clarified large areas of human experience, in their analysis of the functioning and the forces of the physical world around us. This has enabled us to plan and to build, and to control great forces, and no doubt these abilities will grow much greater in the future. So it seems to a scientist a strange question, to be asked what fundamental justification there can be for the validity of his work, when he sees its products operating successfully and developing further, day by day. On the other hand, as Weinberg indicates, most physicists today would consider it unwise to seek within Science the proper basis for philosophy. Science is pragmatic, and we have seen enormous changes of viewpoint within the period of our own lives. All that was established before remains valid through these changes, of course, while becoming only a small part of the greater whole which has subsequently emerged and which continues to emerge. The relationships between scientific truths, and the perspective in our view of them, naturally continue to change as the picture becomes both larger and more detailed. As further knowledge is added, the patterns we see develop and come to have different meanings, and we must change our views in accord with them. Perhaps all this says is that we scientists no longer consider ourselves to be natural *philosophers*, seeking an absolute and final understanding, but see ourselves simply as men trying to comprehend, and to use for further discovery and comprehension, the forces acting within our Universe.

R.H. Dalitz

R. H. Dalitz is Royal Society Research Professor in Theoretical Physics in the University of Oxford, UK.

Errata

- In the review of *Ludwig Wittgenstein: Remarks on the Foundations of Mathematics* (see *Nature*, **281**, 242; 1979), point 39 in the series of quotations from the work should have read:

> 39. The proposition '$a = a$', '$p \supset p$' ['If p, then p'], 'The word "Bismarck" has 8 letters', 'There is no such thing as reddish-green', are all obvious and are propositions about essence: what have they in common? They are evidently each of a different kind and differently used. The last but one is the most like an empirical proposition. And it can understandably be called a synthetic *a priori* proposition.
>
> It can be said: unless you put the series of numbers and the series of letters side by side, you cannot know how many letters the word has.

Also, Michael Dummett, the reviewer, is Wykeham Professor of Logic in the University of Oxford, UK.

- In the review of *Handbook of Geochemistry* (see *Nature*, **281**, 242; 1979), line 6 should have read "Part 5 is also by far the largest to appear,".

Recent scientific and technical books

Biology

ALTMAN, Philip L., and KATZ, Dorothy Dittmer (compiled and edited by). Inbred and Genetically Defined Strains of Laboratory Animals. Part 1: Mouse and Rat. (Biological Handbooks, III.) Pp.xxvi + 418. ISBN-0-913822-12-4. (Bethesda, MD: Federation of American Societies for Experimental Biology, 1979. Distributed in UK by John Wiley and Sons, Ltd., Chichester.) £35.75.

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BINDER, Henry J. (ed.). Mechanisms of Intestinal Secretion. (Krov Foundation Series. Vol.12.) Pp.xiii + 299. ISBN-0-8451-0302-4. (New York: Alan R. Liss, Inc., 1979.) $28.

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APPOINTMENTS VACANT

IMPERIAL COLLEGE
(University of London)
DEPARTMENT OF CHEMISTRY

LECTURESHIP IN INORGANIC CHEMISTRY

Applications are invited for the above post. Candidates should have active research experience and will be expected to develop a vigorous research programme in a field of inorganic chemistry which has sound prospects for future development. Salary in the Lecturer scale £5,073 — £9,732 inclusive of London Allowance.

Applications with curriculum vitae, giving research interests and the names of three referees, to Sir Geoffrey Wilkinson, Department of Chemistry, Imperial College, London SW7 2AY, from whom further particulars may be obtained. Closing date for applications January 31st 1980. 3472(A)

THE CAMPDEN FOOD PRESERVATION RESEARCH ASSOCIATION

GRADUATE ENGINEER

to work on a variety of instrumentation and control problems. In the first instance the work would be primarily concerned with microprocessor applications for the Food Industry.

Industrial or research experience would be an advantage, not necessarily in food technology.

Salary in accordance with age and experience on Research Officer scale within the range £3,048 to £5,316 p.a.

Job specification and application form on request to the Secretary-General, The Campden Food Preservation Research Association, Chipping Campden, Glos. GL55 6LD. 3437(A)

UNIVERSITY OF DUNDEE
DEPARTMENT OF BIOLOGICAL SCIENCES

LECTURESHIP

Applications are invited from geneticists, microbiologists, molecular biologists and biochemists for the post of Lecturer in the Department of Biological Sciences.

Experience of nucleic acid or microbial genetic techniques would be an advantage though not essential.

Salary scale £4,333 to £8,992 (under review).

Applications (6 copies, or if posted overseas 1 copy) including a Curriculum Vitae and the names and addresses of two referees should be sent by 23 October 1979 to the Secretary, The University, Dundee, DD1 4HN from whom further details of the post can be obtained. Please quote Ref: EST/82/3/79. 3422(A)

Head of Pharmaceutical Development

We wish to appoint a Head of Pharmaceutical Development at our Pharmaceutical Research Laboratories. The person appointed will be a qualified pharmacist with several years' experience in the industry and will take charge of a young and successful department of some 20 staff which is responsible for the development of new drug formulations, the supply of test compounds for human and animal trials and basic pharmaceutical research.

This is a key senior appointment in the Research Group of a major international company and will appeal to a creative pharmacist with a proven record of success in research, who is now seeking a greater challenge. Salary and other benefits – including a company car – will reflect the importance of the position.

The modern and exceptionally well-equipped laboratories are situated in the new city of Milton Keynes in a pleasant part of North Buckinghamshire.

Scientists, male or female, whose experience meets our requirements are invited to send a brief curriculum vitae to:

Dr. J. D. Coombes, Research Director,
Hoechst Pharmaceutical Research Laboratories,
Walton Manor, Walton, Milton Keynes MK7 7AJ.

Hoechst

3431(A)

UNIVERSITY OF WESTERN AUSTRALIA
Perth

LECTURESHIP IN PHYSICAL AND INORGANIC CHEMISTRY

Applications for the above position in th Department of Physical and Inorgani Chemistry are invited from suitabl qualified persons. The appointee will b required to share in the genera undergraduate teaching responsibilities o the Department, to lecture in his fields o specialisation at undergraduate an postgraduate levels and to carry ou research for which time and good facilitie are available.

The School of Chemistry occupies a we equipped building with machine, electroni and glass-blowing workshops. A wid range of equipment for physical measure ments is available, and the University ha good computing facilities. The staff of th Department consists of seventeen member and there are 35 postgraduate an postdoctoral research workers. Furthe details of the Department's curren research interests and publications ar available on request from the Head.

The current salary range for a Lecture is: $A16,291 to $A21,401 per annum Benefits include superannuation similar t FSSU, fares to Perth for appointee an dependent family, removal allowanc study leave and long service leave an housing loan scheme.

Applications in duplicate stating fu personal particulars, qualifications an experience should reach the Actin Staffing Officer, University of Wester Australia, Nedlands, 6009, Wester Australia, by 10th November, 197 Candidates should request three referees t write immediately to the Acting Staffin Officer. 3478(A)

THE UNIVERSITY OF SYDNEY

LECTURESHIP IN BIOLOGY

Applications are sought from persons wi a research interest in any field of vertebra biology. Preference may be given applicants in the fields of development biology, physiology, behaviour, ecology evolution. Appointee will be expected carry out and supervise research in his her special field, to participate in teachi of vertebrate biology and to contribute general teaching programme of the Scho

The position is expected to be filled by probationary appointment of three yea capable of leading to tenure but if all t University's requirements for tenure a deemed to be satisfactorily met tenure m be granted at the time of appointment.

Salary range: $A16,291 to $21,401 p.

Applications including curriculum vita list of publications and names of th referees by 9 November 1979 to Registr University of Sydney, NSW 20 Australia, from whom further informati available. Information also available fr the Association of Commonwea Universities (Appts), 36 Gordon Squa London WC1H 0PF. 3476(A

Editor of Nature

Applications are invited from those wishing their names to be considered for this appointment, which will become vacant at the end of this year.

A brief account of past career, qualifications and experience considered relevant should be sent in writing to:

nature

The Publishing Director,
Macmillan Journals Limited,
4 Little Essex Street,
LONDON WC2R 3LF

3456(A)

JOHN INNES INSTITUTE

Applications are invited for a three year temporary appointment in the

DEPARTMENT OF GENETICS

To collaborate with a group working on the genetics of the nitrogen fixing symbiosis between *Rhizobium* spp. and legumes by complementing their genetical studies with the physical analysis of plasmids which carry genes essential for nodulation and nitrogen fixation. Although the group's work will continue, this appointment is to initiate physical studies on plasmids and this particular aspect is expected to be completed by the end of the three year period. Further particulars obtainable from Professor D.A. Hopwood, Head of the Department of Genetics.

Applicants should have a first or upper second class honours degree in biochemistry, microbiology, molecular biology or genetics, with at least two years' post-qualifying experience, but preferably PhD and relevant postdoctoral experience. Appointment as Higher Scientific Officer, salary w.e.f. 1.1.80 £5,097 to £6,737). Starting salary epending on experience. Non-contributory superannuation.

Applications, quoting reference GEN/33, giving full particulars and the names of two referees, should be sent to the Secretary, John Innes Institute, Colney Lane, Norwich, NR4 7UH, not later than th November, 1979. 3469(A)

ST HELIER HOSPITAL
Carshalton, Surrey
POST DOCTORAL RESEARCH ASSISTANT

Required with experience in Immunology or Enzymes. Small department with 3 other workers.

Reply with Curriculum Vitae and names of three referees to Dr E.L. Rhodes at above hospital by 27th October.

3467(A)

BLOOD GROUP REFERENCE LABORATORY
SENIOR SCIENTIFIC OFFICER

The Blood Group Reference Laboratory is a Central Reference Laboratory for the National Blood Transfusion Service. This laboratory is a substantial producer of blood grouping reagents of human and animal origin. In the near future significant changes in manufacturing procedures are foreseen with changes in processing, the use of hyperimmune donors and *in vitro* cultures of lymphocytes as new sources of material. It is anticipated that this laboratory will be increasingly involved in monitoring quality of grouping reagents from a variety of external sources.

A Senior Scientific Officer is sought to take charge of all aspects of production and quality control of blood grouping reagents and to be responsible for new developments and research in these areas. Salary: £8,182 to £10,097 (increase pending) plus £502 L.W. Further information may be obtained from Dr A.M. Holburn, 01 730 2152.

Application in writing with details of experience and the names of two referees to: Mis K Lomas, Blood Group Reference Laboratory, Gatliff Road, off Ebury Bridge Road, London SW1W 8QJ.

3440(A)

THE CAMPDEN FOOD Preservation Research Association, Chipping Campden, Glos GL55 6LD, requires a food scientist, food technologist or home economist to head the quality appraisal team in its Department of Agriculture and Quality. The successful candidate will be enthusiastic, have qualities of leadership and get on well with people. Salary in the Research Officer (£3,048 to £5,316) or Senior Research Officer grade (£4,884 to £7,260). Further details and application form from the Secretary-General at the above address. 3436(A)

WELSH NATIONAL SCHOOL OF MEDICINE
(University of Wales)
DEPARTMENT OF SURGERY

Applications are invited from post-doctoral scientists with wide experience and proven capability in the field of cellular immunology for the post of Lecturer in the above department at Heath Park, Cardiff. Special expertise in the field of cancer or gastrointestinal immunology would be advantageous. The successful candidate will direct the scientific side of a clinically orientated research laboratory, working in close collaboration with clinicians with similar interests. The appointment will be for 3 years in the first instance with possible renewal thereafter to be discussed with interested candidates.

Salary on the scale for Non-Clinical Lecturers (£4,333 to £8,922 per annum, under review); starting point dependent upon qualifications and experience. U.S.S. available.

Further particulars available from the Registrar and Secretary, Welsh National School of Medicine, Heath Park, Cardiff, CF4 4XN, to whom applications should be submitted 3 weeks from the date of the appearance of this advertisement.

3444(A)

KINGSTON POLYTECHNIC
SCHOOL OF GEOLOGY
SLIDE MAKER

To be responsible for all thin section preparation for the School, together with such ancillary techniques and preparation of polished ore specimens, specimen slabbing, and polishing and preparation of grain slides.

Experience essential: Advanced Technician Certificate an advantage. T3/4 £4,470-£5,457 inclusive.

Application forms from Assistant Registrar (Personnel), Kingston Polytechnic, Penrhyn Road, Kingston upon Thames KT1 2EE. Tel: 01-549 1366.

3441(A)

STANFORD UNIVERSITY
GEOCHEMIST —PETROLOGIST

The Department of Geology in the School of Earth Sciences invites applications for a faculty position beginning September, 1980. Areas of interest include (but are not limited to) geochemical processes in the crust and mantle of the earth including isotope geochemistry, thermodynamics, kinetics, element distribution and phase equilibria studies. Teaching responsibilities might include graduate and undergraduate courses in geochemistry and igneous petrology. Level of appointment is open, but we are particularly interested in a creative young person, with a well-established record in research.

Applications should include (1) a resume of education, professional experience, and published research; (2) a brief statement of plans for future research, and (3) the names of 4 referees. Send to: Prof. J.G. Liou, Department of Geology, Stanford University, Stanford, CA 94305, by December 1st, 1979. Stanford University is an equal-opportunity/affirmative-action employer. W329(A)

BLOOD GROUP REFERENCE LABORATORY
SCIENTIFIC OFFICER

This laboratory is a Central Reference Laboratory for the National Blood Transfusion Service. A graduate is sought for research and development of techniques for the detection of antibodies to neutrophil leucocytes. Experience in immunology or immunohaematology desirable.

Application in writing with details of experience and the names of two referees to: Miss K. Lomas, Blood Group Reference Laboratory, Gatliff Road, off Ebury Bridge Road, London SW1W 8QJ.

3439(A)

Murdoch University

PERTH, WESTERN AUSTRALIA

DEANSHIP OF THE SCHOOL OF VETERINARY STUDIES

(Ref: EN0007)

Applications are invited from suitably qualified persons for appointment to the position of Dean of the School of Veterinary Studies which has become vacant owing to the appointment of Professor R H Dunlop to the University of Minnesota, U.S.A. The position is available from 1 February 1980 or as soon thereafter as possible.

The qualifications expected of applicants include a higher degree, substantial experience in veterinary education and eligibility for registration under the Western Australian Veterinary Practitioners Act. The successful applicant will be appointed both as Dean of the School for a period of five years, which may be renewed at the discretion of the governing body and as a permanent full-time professor in the applicant's particular discipline.

Although the School is an integrated unit, it is administered as two divisions. Veterinary Biology is headed by Professor J. Mc. Howell and Applied Veterinary Medicine by Professor M.E. Nairn. R.G. Wales is the Professor of Physiology, R.A. Swan is the Professor of Veterinary Clinical Studies, W.T. Clark is the Professor of Small Animal Medicine and Surgery and the present establishment for academic staff is 39. There is an entry quota of forty-eight students per year. The initial developmental phase of the School is now completed and the first major graduation of Bachelor of Veterinary Medicine and Surgery students will take place early in 1980.

SALARY AND CONDITIONS The salary is at present $A34,119 per annum. Conditions of appointment include superannuation similar to F.S.S.U., long service leave, access to outside studies programme, payment of fares to Perth for appointee and dependent family, removal and settling-in allowances and house purchase scheme.

PROCEDURE FOR APPLICATION There is no prescribed application form, but TWO COMPLETE SETS of detailed applications, quoting the appropriate reference number, including full personal particulars, details of tertiary qualifications career history with description of posts held, area of special competence and interest, research completed or currently being undertaken, membership of professional institutions or societies and positions of responsibility in these, list of relevant material published by the applicant, when available to take up appointment if offered and the names and addresses of three professional referees should reach the Personnel Officer, Murdoch University, Murdoch, Western Australia 6150 by *5 November 1979.*

Applicants resident in the United Kingdom, Europe or Africa at the time of application should also forward ONE further copy to:—

The Secretary General
Association of Commonwealth Universities (Appts)
36 Gordon Square
London WC1H 0PF

Further general information on the University and the Conditions of appointment and facilities is available from the Personnel Officer, Murdoch University or from the Association of Commonwealth Universities. 3455(A)

THE POLYTECHNIC — HUDDERSFIELD

RE-ADVERTISEMENT

(Previous applicants need not re-apply)

DEPARTMENT OF LIFE SCIENCES

PRINCIPAL LECTURER, SENIOR LECTURER OR LECTURER II — HUMAN ECOLOGY

Ref: ACA/343/A/N

Preference will be given to candidates with teaching and/or industrial experience and a proven research record and possessing a first degree in a Biological subject and a higher degree in Agriculture or Biological resource management. Experience of developing countries particularly in tropical resources would be advantageous.

Staff are expected to undertake activities, including research, in addition to teaching duties.

Salary	PL	£7,680 — £8,523 (Bar) — £9,639
	SL	£6,597 — £7,710 (Bar) — £8,253
	LII	£4,770 — £7,149 per annum

Further details and application forms, which should be returned by 26th October, 1979 from the Personnel Office, the Polytechnic, Queensgate, Huddersfield, HD1 3DH (Telephone 0484 22288 Ext 2223). Please quote the relevant reference number. 3463(A)

UNIVERSITY OF RHODESIA

PROFESSOR AND HEAD OF DEPARTMENT OF ZOOLOGY

Candidates should hold a doctorate in Zoology, should have experience in research and teaching at university level, and should have held a senior university appointment in Zoology.

LECTURESHIP/SENIOR LECTURESHIP IN GEOLOGY

Applicants must hold, at least, an honours degree in Geology, with experience in metamorphic and/or sedimentology and coal geology.

Salary scales and conditions of service: Professor £9,162×345 — 10,872 p.a. Senior Lecturer £6,662×259 — 7,439 ×274 — 8,809 p.a. Lecturer Grade I £6,043×227 — 6,951 p.a. Lecturer Grade II £3,731×196 — 4,711×227 — 5,846 p.a. (approximate conversions from Zimbabwe-Rhodesia $ to £ sterling at 7/9/79.)

Permanent Pensionable Terms: Family passages and allowance towards transport of effects on appointment. Installation loan of up to half of one year's salary if required. Unfurnished University accommodation guaranteed for a period of at least three years for persons recruited from outside Zimbabwe-Rhodesia. Sabbatical leave and biennial visits with travel allowances. Superannuation and medical aid schemes.

Short-Term Contracts: Family passages and allowance towards transport of effects. Assistance with accommodation for persons recruited from outside Zimbabwe-Rhodesia.

Applications (6 copies) should give full personal particulars (including full name, place and date of birth, etc.), qualifications, experience, publications, present salary, date of availability, and names and addresses of three referees, and should be sent to the Appointments Officer (Staff), University of Rhodesia, PO Box MP 167, Mount Pleasant, Salisbury, Zimbabwe-Rhodesia. Please state clearly for which post application is being made. Overseas applicants should send an additional copy of their application to the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, from whom conditions of appointment can be obtained.

Closing date for applications — *15 November, 1979.*

IMPORTANT British subjects considering applying for posts in Zimbabwe-Rhodesia are urged to consult the Foreign and Commonwealth Office (tel: 01-233 4995) or their nearest British Consular Office. 3426(A)

THE UNIVERSITY OF MANITOBA

invites applications for the position of

PROFESSOR AND HEAD of the DEPARTMENT OF STATISTICS

Currently there are twelve faculty members in the Department which offers undergraduate and graduate programs, and the laboratories and facilities provide a stimulating environment for research and teaching.

Salary will be appropriate to the qualifications of the appointee. The 1979-80 floor for full professors is $29,743.

Canadian citizens, landed immigrants and others eligible for employment in Canada at the time of application are especially encouraged to apply.

Applications should be made as soon as possible, giving curriculum vitae, publication list and names of three referees to Dr C C Bigelow, Dean, Faculty of Science, University of Manitoba, Winnipeg, Manitoba, R3T 2N2.

W417(A)

THE AUSTRALIAN NATIONAL UNIVERSITY

Applications are invited from suitably qualified persons seeking appointment to the following positions.

RESEARCH SCHOOL OF BIOLOGICAL SCIENCES

DEPARTMENT OF ENVIRONMENTAL BIOLOGY

FELLOW

The Department wishes to make a tenured appointment in experimental community ecology. Applicants should be experienced in dynamic aspects of community ecology (plant demography or plant populatior biology). Familiarity with theoretica community ecology is desirable but we seek applicants who will devise experiments tc test key concepts in community theory. It is desirable that such a research programme should complement the Department's interests in physiological plant ecology.

Closing date: 16 November, 1979.

DEPARTMENT OF GENETICS

FELLOW IN MOLECULAR GENETICS

The research project for which this post ha been established will require the successfu applicant to collaborate with Dr B. Rolf on work involving the genetic manipulation of nitrogen fixation genes i *Rhizobium*. According applicants shoul have completed at least 5 years relevan postdoctoral research and have experienc of a wide range of recombinant DN techniques.

Closing date: 16 November, 1979.

RESEARCH SCHOOL OF PHYSICAL SCIENCES

DEPARTMENT OF MATHEMATICS

RESEARCH FELLOW

Current departmental research interes include number theory, group theor (computational group theory, Burnsic problems, groups of prime-power orde and varieties), global analysis an harmonic analysis, ordinary differenti equations and control theory, foundatio and set theory, linear algebraic groups, ar combinatorics, but applications will considered in any field of moder mathematics.

Closing date: 10 November, 1979.

Conditions of appointment: Salaries w be in accordance with qualifications ar experience within the range: Fello $A18,990 to $25,441; Research Fello $A16,291 to $21,265 per annum. Prese exchange rate $A1 = UK 50p.

Appointment as Research Fellow Mathematics is for three years with t possibility of extension to five years. Fellow is appointed for five years in the fi instance with the possibility reappointment after review to age 65.

Reasonable appointment expenses a paid. Superannuation benefits a available for applicants who are eligible contribute. Assistance with findi accommodation is provided for appointee from outside Canberra. T University reserves the right not to make appointment or to make an appointme by invitation at any time.

Prospective applicants should fi obtain further particulars from t Association of Commonweal Universities (Appts), 36 Gordon Squa London WC1H 0PF. 3429(A

INSTITUT FÜR PHYSIOLOGISC Chemie der Universität, Hambu Application invited for two positions. Technische/r Angestellte/r and 1 W Angestellte/r). Research in cellu differentiation, cell membranes, pro synthesis. Letter of application to Prof Gebhard Koch, Physiologisch-Chemisc Institut, Abteilung Molekular-biolo Grindelallee 117, 2000 Hamburg Germany. W427(A

Centre for Overseas Pest Research, Kensington, London

Field Entomologist

The Centre for Overseas Pest Research seeks to help developing countries by devising efficient pest control techniques.

The successful candidate will be involved in work overseas on projects concerned with research surveys, control and training activities related to major crop pest problems.

The person appointed can expect to spend a considerable proportion of time on overseas tours, which are likely to be in Eastern Africa for the first few years.

Applicants, usually under 32 years of age, should have a good honours degree in a biological subject, with some training in entomology, and at least 4 years' postgraduate experience, preferably including several years' overseas experience working on major pest problems in developing countries.

Appointment as Senior Scientific Officer £6,655 – £8,640 (to become £7,110 – £9,485 from 1.1.80) with starting salary according to qualifications and experience. Promotion prospects. Non-contributory pension scheme.

For further details and an application form (to be returned by 5 November 1979) write to Mr H Woodman, Centre for Overseas Pest Research, College House, Wrights Lane, London W8 5SJ.

Please quote ref: SA/54/JA.

Ministry of Agriculture, Fisheries and Food,
Radiobiological Laboratory, Lowestoft

Scientists/Engineers

Each licensed nuclear site disposing of liquid radioactive wastes in the environment requires a discharge assessment before MAFF, as authorising department, can decide on the permissible level of discharge. All new sites require such an assessment, and discharges from existing sites require periodic examination.

Assess the effect of radioactive wastes on the environment

The successful candidate will advise senior staff of permissible discharge limits on the basis of information obtained from assessing the relationship between the discharge rate and the extent of radiation affecting individuals or critical groups within the population. This calculation will also involve estimating the collective dose to the population as a whole. In addition, the work will include developing and applying new methods for evaluating waste restrictions, maintaining a close liaison with research staff and participating in national and international working groups.

Candidates, normally aged under 30, should have a good honours degree in physics, chemistry or engineering. At least 2 years' postgraduate experience essential. Some experience in OR environments advantageous.

Appointment as Higher Scientific Officer. Starting salary (according to qualifications and experience) within the range £4,725 – £6,210 (to become £5,095 – £6,735 from 1.1.80). Promotion prospects. Non-contributory pension scheme.

Assist with radiological control programmes

The successful candidate will join a team responsible for the design, development, execution and interpretation of environmental monitoring programmes. In addition to playing a full role in this work, the scientist appointed will assist with the collection, preparation, interpretation and analysis of information, to ensure that existing and future monitoring programmes are run effectively and productively. Field studies, including work on research vessels will be an important aspect of the job.

Candidates, normally aged under 27, should have a good honours degree or equivalent in physics, chemistry or biology. An ability to handle data and apply techniques of numerical analysis essential.

Appointment as Scientific Officer. Starting salary (according to qualifications and experience) within the range £3,285 – £5,085 (to become £3,590 – £5,485 from 1.1.80). Promotion prospects. Non-contributory pension scheme.

For further details and an application form (to be returned by 2 November 1979) write to Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, or telephone Basingstoke (0256) 68551 (answering service operates outside office hours).
Please quote ref: SB/48/AD. 3468(A)

ALL THESE POSTS ARE OPEN
TO MEN AND WOMEN

Science Group
CIVIL SERVICE

vana

incipal Hydrological Engineer

Salary up to £10,210 plus allowances

Candidates must possess a degree in science or engineering plus a post-graduate qualification in Hydrology and preferably have 12 years' field and office experience. (Applicants with less experience may be considered for appointment at a lower salary scale).

The duties of the post include the operation and minor expansion of hydrometric networks in the Okavango Delta and on several ephemeral rivers, data processing, evaporation studies and water resources planning, including the supervision of consultants.

Salary includes a substantial tax-free allowance paid under Britain's overseas aid programme. Basic salary attracts 25% tax-free gratuity.

Benefits include free passages, generous paid leave, children's holiday visit passages and education allowances, appointment grant and interest-free car loan.

The terms on which civil and public servants may be released if selected for appointment will be subject to agreement with their present employers.

For full details and application form write quoting MA/733/NA.

Crown Agents

The Crown Agents for Oversea Governments and
Administrations, Recruitment Division,
4 Millbank, London SW1P 3JD.

3447(A)

THE UNIVERSITY OF NEW ENGLAND

Armidale, New South Wales

DEPARTMENT OF AGRONOMY & SOIL SCIENCE

LECTURESHIP IN SOIL SCIENCE

The Division of Soil Science within the Department of Agronomy and Soil Science is responsible for undergraduate teaching of Soil Science in the Faculties of Rural Science, Resource Management, Arts and Economic Studies. Research and postgraduate training within the Division is concerned with various aspects of soil chemistry, biology and physics in relation to soil genesis, fertility and management.

The appointee will lecture and organise practical classes in introductory Soil Science courses. Supervision of advanced student projects will also be required. An appointee is sought in the areas of Soil Biology and/or Soil Management (soil conservation, pollution, reclamation, etc.) and preference will be given to persons in either of these areas having training in Soil Science and experience in teaching and research. The appointee is expected to take up duties in February 1980.

Salary: $A16,291 – $21,402 p.a. Assistance will be given with travel and removals.

Applicants in the UK, Europe and America should forward their applications, and ask their referees to forward their reports, without delay to the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, from whom conditions of appointment can be obtained. An additional copy of application only should be sent to the Staff Officer, University of New England, Armidale, NSW 2351, Australia, quoting Position No. 424.

Applications close *31 October, 1979.* 3477(A)

UNIVERSITY OF DUBLIN
Trinity College

RESEARCH ASSISTANT IN GEOLOGY

A Research Assistant is required for the newly established Applied Geology Unit at Trinity College Dublin. The successful candidate will join a joint Trinity College/Geological Survey of Ireland team making an integrated stratigraphical study of offshore Mesozoic basins based upon material obtained from petroleum exploration wells. The post, available in January 1980, will be for a period of 3 to 4 years.

Salary scale: £3,174 to £4,286 p.a. Appointment will be made at a point commensurate with the qualifications and experience to date of the successful candidate.

Applicants, who should have a degree in Geology and interests in Micropalaeontological and palynological techniques, should send a curriculum vitae and the names of two referees to Professor C.H. Holland, Department of Geology, Trinity College, Dublin 2, to arrive by November 12th 1979. 3445(A)

UNIVERSITY OF DUNDEE

DEPARTMENT OF BIOCHEMISTRY

POSTDOCTORAL RESEARCH FELLOW

Applications are invited for a post doctoral research post (MRC funded) to study the regulation of the synthesis of anaerobic enzymes in *Escherichia coli*. Ideally the successful candidate will have had some previous experience of the techniques of DNA sequencing and/or *in vitro* protein synthesis.

The appointment is tenable for one year initially, with the possibility of extension for a further two years at a starting salary of up to £5,193 (under review) depending upon experience.

Informal enquiries may be made to Dr. B. A. Haddock, Department of Biochemistry, University of Dundee, Dundee DD1 4HN (telephone 0382 23181 ext. 551).

Applications including curriculum vitae and the names and addresses of two referees should be sent to The Secretary, The University, Dundee DD1 4HN quoting Reference EST/84/79X as soon as possible. 3423(A)

UNIVERSITY OF OXFORD

DEPARTMENT OF AGRICULTURAL SCIENCE

POST DOCTORAL RESEARCH ASSISTANT

Applications are invited for a three year project funded by ARC to work with Dr B C Loughman on the metabolism of phenoxyacetic acid derivatives in higher plants and possible relevance to selective action of these and other herbicides. The isolation of hydroxylating, glycosylating and polymer forming enzyme systems and investigation of possible mechanisms of control by light and other factors will be major aspects of the work.

The starting date will be 1 January, 1980 and the salary in the Grade 1A range (£4,333 to £7,521). Applicants, who should be familiar with enzymological methods and have experience with plant material, should write to the Administrator, Department of Agricultural Science, Parks Road, Oxford OX1 3PF enclosing curriculum vitae and names of two referees by 31 October. 3475(A)

RESEARCH SCIENTISTS BIOLOGICAL SCIENCES

Chicago, Illinois USA Positions

The Life Sciences Division of IIT Research Institute, a not-for-profit Chicago, Illinois, USA based research organization has several staff positions for biologists. Requisite are PhD in physiology, toxicology, nutrition or experimental pathology. Preference will be given to candidates with post doctoral research experience and interest in long-term toxicity, carcinogenicity bioassay and carginogenesis studies in rodents. Opportunity is available to develop independent research projects in own field of expertise. Salary commensurate with experience and qualifications.

Please submit resumes including salary history to:

Ms. L. Amft
IIT
Research Institute
10 W. 35th St.
Chicago, Illinois, USA 60616

IITRI is an Equal Opportunity/Affirmative Action Employer and encourages applications from women and members of minority groups. W418(A)

MEDICAL RESEARCH COUNCIL

CLINICAL ONCOLOGY & RADIOTHERAPEUTICS UNIT

CANCER TRIALS OFFICE

Executive Officer/Computer Programme required to help in the co-ordination o national clinical trials in the treatment o cancer. The work will include responsib ility for the collection of data (involvin liaison with hospital workers, mainly b telephone or letter), the organisation o office records and computer programming. Programming will probably occup about half working hours.

The post, which is available for thre years in the first instance, is suitable fo someone with organisational ability experience in computer programming an an interest in medicine.

Salary on scale from £3,184 pa at age 2 (£3,450 from 1.1.80).

Please apply to the Administrativ Officer, MRC Clinical Oncology Radiotherapeutics Unit, The Medic School, Hills Road, Cambridge CB2 2Q 3446(A)

THE UNIVERSITY OF MANCHESTER

RESEARCH ASSOCIATE IN PATHOLOGY

Applications invited for this post, tenab for three years from a date to be arrange The project is to investigate the nature a significance of intraduct proliferati breast lesions and their relationship carcinoma in humans.

A variety of techniques will be employ including electron microscopy, microdensitometry and immunofluorescence.

Initial salary range p.a.: £4,333 £4,910. Superannuation.

Applications (Curriculum vitae a three referees) should be sent to t Registrar, The University, Manches M13 9PL by October 19th. Quote r 221/79/N. Further information from Dr Harris, Department of Patholog University Hospital of South Manchest Manchester, M20 8LR (Tel: 061 445 81 ext 2760). 3448(A)

POSTDOCTORAL POSITION availa immediately for research on biochemis of membrane transport in plant cells. T position is for one year, with possi extension, at a salary of $11,700 to $12,9 p.a. Send c.v. with names, addresses a telephone numbers of two or three refere to Ronald Poole, Biology Departme McGill University, 1205 Ave. Doct Penfield, Montreal, Quebec H3A 1 Canada. W421(A)

THE DEPARTMENT OF
MICROBIOLOGY
at

SOUTHWESTERN MEDICAL SCHOOL

is soliciting applications for a junior (tenure-track) or senior

FACULTY POSITION

in the area of
MOLECULAR IMMUNOGENETICS

Candidates should have a PhD or quivalent, postdoctoral training, and rovide evidence for substantial ontributions in this field. Appointee will e expected to participate in teaching rograms for medical and graduate tudents and establish an active research rogram.

Send curriculum vitae and the names of 3 eferences to: Dr. James Forman, Department of Microbiology, outhwestern Medical School, 5323 Harry lines Blvd., Dallas, Texas 75235. An ffirmative action/equal opportunity mployer. W425(A)

MANCHESTER AREA HEALTH AUTHORITY

South District
Withington Hospital
Nell Lane, Manchester
M20 8LR

POST PROBATIONARY BIOCHEMIST

equired to work in the Department of rology under the direction of the rofessor of Urology. The research work ill involve the analysis of prostatic cretion in various types of prostatic sease and the development of radio nmunoassay of specific prostatic nzymes.

A main interest in this work is the early etection of neoplasia, a good honours egree in biochemistry is essential and pplicants should preferably also have a gher degree.

Job description and application form vailable from Staff Office. Tel: 061 445 11 Ext. 2265. Closing date 12th October, 979. 3421(A)

THE AUSTRALIAN NATIONAL UNIVERSITY

RESEARCH SCHOOL OF
PHYSICAL SCIENCES

CHAIR OF MATHEMATICS

ne University is seeking to appoint a rofessor to a Chair of Mathematics in the esearch School of Physical Sciences thin the Institute of Advanced Studies. ne successful appointee will, in ordinary rcumstances, be expected to accept pointment as Head of the Department r an initial term in accordance with rrent practice relating to the headship of partments in the Institute, with the ssibility of reappointment for one or ore further terms. Appointment as ofessor is to retiring age (at present 65), th an option to retire after the age of 60. Salary will be not less than $A34,119 per num. Current exchange rates are 1 = $US1.12 = 50p.

Applications are invited from persons in y field of mathematics. This is a position voted to research and graduate training d there are no obligatory undergraduate ching responsibilities.

The University reserves the right not to ke an appointment or to make an pointment by invitation at any time.

Intending applicants should, in the first tance, obtain the further particulars m the Association of Commonwealth iversities (Appts), 36 Gordon Square, ndon WC1H 0PF. Closing date: 1 April, 80. 3428(A)

OVERSEAS DEVELOPMENT

KNOW-HOW-vital to developing countries

Chief Extension Officer
Senior Research Officer Sudan

The Southern Region Agriculture Development Project is entering a considerably expanded 4 year phase with multi-donor financing and the British Government has undertaken to provide a substantial number of senior and supporting staff for the project.

The Chief Extension Officer will head the Rural Services Division, Juba and be responsible for extension and nutrition services, crop marketing, support media activities and training. Appropriate degree with a minimum of 10 years relevant post-graduate experience required with sound knowledge of extension in small-holder agriculture in Africa and some experience in operation of credit schemes and marketing.

The Senior Research Officer will lead the Crop Research Unit, Yei and be responsible for the planning, execution and supervision of the research programme concentrating on the important food crops of higher rainfall areas; establishment of pest, disease and weed control service; development of improved farming systems. Appropriate degree with minimum of 10 years experience in agricultural research and development in tropical areas required with emphasis on food crop production in rainfall conditions.

Applicants should be citizen of the United Kingdom aged 35 to 50 years and should be prepared for extensive travel within the region.

Salary in senior range above £10,000 pa (UK taxable) plus variable tax free overseas allowances £1,040 to £3,175 pa £425 to £850 pa depending on marital status.

Appointments for 2 years with home leave after 12 months. Further posts of Entomologist, Farm Management Specialist, Senior Extension Officer and Agronomist will arise as housing becomes available over the next year.

These posts are wholly financed by the British Government under Britain's programme of aid to the developing countries. Other benefits normally include paid leave, free family passages, childrens education allowances and holiday visits, free accommodation and medical attention.

For full details and application form please apply, quoting ref (331X) stating post concerned, and giving details of age, qualifications and experience to:—

Appointments Officer,
OVERSEAS DEVELOPMENT ADMINISTRATION,
Room 301, Eland House,
Stag Place, London SW1E 5DH.

ODA

HELPING NATIONS HELP THEMSELVES

3425(A)

UNIVERSITY OF LEICESTER

DEPARTMENT OF
MICROBIOLOGY

TECHNICIAN

or Biology Sciences graduate required to assist in research work on the genetics and manipulation of Influenza viruses. A sound background in molecular biology and/or experience in tissue culture techniques would be an advantage. Applicants should have obtained an O.N.C. or equivalent qualification. The successful applicant will be encouraged to take an active interest in research projects.

Salary on the Grade 3 scale £3,122 to £3,553 a year.

Please write for application forms to Mr. M. Stevens, Department of Microbiology, The University of Leicester, University Road, Leicester, LE1 7RH. 3434(A)

UNIVERSITY OF BRADFORD

POSTGRADUATE SCHOOL OF PHYSICS
RESEARCH ASSISTANT

Applications are invited for the above 3 year post which entails collaboration with academic staff in the design, implementation and commissioning of the control system for a large magnetic spectrometer to be installed at the SRC Laboratory at Daresbury in Cheshire. The successful applicant's prime area of responsibility will be to write programmes for control and running of the spectrometer in real time.

The successful candidate will be a graduate in a relevant field (Physics, Electrical Engineering, Computing) and experience in real time programming would be an advantage. Initial salary within the range £3,775 to £4,910 according to age, experience and qualifications.

Application forms obtainable from the Registrar Post Ref: PY/RA/90/X, University of Bradford, Bradford BD7 1DP. 3459(A)

ASSISTANT PROFESSOR

We have an opening at the assistant professor level in the
DEPARTMENT OF MICROBIOLOGY

at the

UNIVERSITY OF BRITISH COLUMBIA

We are seeking an individual with training in any aspect of molecular biology. Responsibilities would include teaching at the undergraduate level, direction of graduate students and development of a strong research program. There are several active molecular biology groups at U.B.C. with which the individual may interact.

Please send curriculum vitae and the names of three referees to Dr J.J.R. Campbell, Professor and Head, Department of Microbiology, University of BC, 2075 Wesbrook Mall, Vancouver, BC V6T 1W5. W420(A)

ROCHE RESEARCH INSTITUTE OF MARINE PHARMACOLOGY

BIOCHEMIST

POST-DOCTORAL FELLOW

The Roche Research Institute of Marine Pharmacology has been established at Dee Why, N.S.W., to carry out multidisciplinary research into the biology, microbiology, pharmacology and chemistry of a wide range of natural products from marine organisms.

A biochemist is required to investigate biosynthetic pathways in several eukaryotic microorganisms, including algae. A knowledge of molecular genetics, chemistry and biochemistry of isoprenoids and techniques for studying membrane-bound enzyme systems would be helpful. Experience with HPLC separation techniques would be valuable.

This is a post-doctoral position, initially for 1 year with possible extension to 2 years.

Salary will be negotiated according to experience. Assistance with removal expenses is envisaged.

Applications and names and addresses of three referees should be sent to:

The Director, Roche Research Institute of Marine Pharmacology, PO Box 255, DEE WHY. AUSTRALIA. 2099.

Applications close 31st October, 1979.

3419(A)

Universiteit van Amsterdam

Research/Teaching Position

The Division of Pharmaceutical Chemistry of the Subfaculty of Pharmacy has positions available for

postgraduate research workers (m/f)

These positions are tenable for a maximum of four years during which time the appointee will undertake research leading to the degree of Doctor of Philosophy. The appointee will be engaged in teaching and organizational duties for approximately one third of the time.

Research will be conducted into the physicochemical properties of drugs, with an emphasis on novel formulations and analysis, under the supervision of Dr. E. Tomlinson. Preference will be given to pharmacy and chemistry graduates. The appointments will be made according to the directions laid down by the Board of the University.

Application and curriculum vitae should be sent to:

Dr. E. Tomlinson
Vakgroep Farmaceutische Chemie
Plantage Muidergracht 24
1018 TV Amsterdam, The Netherlands.

Telephone: (020) 522 2241/2252.

W423(A)

RE-ADVERTISEMENT

UNIVERSITY COLLEGE OF NORTH WALES

Bangor

RESEARCH OFFICER IN STATISTICS

Applications are invited for the above appointment in the College's Faculty of Science, to be responsible to the Dean of the Faculty through the Chairman of the School of Mathematics and Computer Science. The duties of the post are primarily to carry out an inspection of statistical packages and their documentation, and to assist in the teaching of statistics and the provision of statistical advice.

Applicants should hold an honours degree in mathematics, statistics or cognate subjects, and have had appropriate experience.

The appointment, to commence as soon as possible, will be for two years, and will be to the lower portion of the Research and Analogous IA scale:— £4,333 to £7,521 per annum.

Applications (two copies), giving details of qualifications and experience, together with the names and addresses of three referees, should be sent to the Assistant Registrar (Personnel), University College of North Wales, Bangor, Gwynedd LL57 2DG, from whom further particulars may be obtained. Closing date for applications: 29th October, 1979. 3415(A)

THE UNIVERSITY OF THE SOUTH PACIFIC

Fiji

Applications are invited for the

CHAIR IN BIOLOGY

(Post 79/86).

Applicants should have proven University teaching and research ability in any one of the disciplines of Marine Biology, Terrestrial and Angiosperm Botany, Community and Population Ecology, Vertebrate Zoology or Ethology for this post. Salary scale; F$19,008 to F$20,420 pa (£1 sterling = F$1.79).

The British Government may supplement salary by £5,334 pa (sterling) for married appointee or £3,192 pa (sterling) for single appointee (reviewed annually and normally free from tax) and provide children's education allowances and holiday visit passages. Family passages; baggage allowance; 3 year contract; 15% gratuity; superannuation scheme; appointment allowance; partly furnished accommodation of maximum rental of 12½% of salary (under review).

Detailed applications (2 copies) with curriculum vitae and naming 3 referees to be sent direct to the Registrar, University of the South Pacific, PO Box 1168, Suva, Fiji by 16 November, 1979. Applicants resident in the UK should also send one copy to Inter-University Council, 90-91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address. 3427(A)

UNIVERSITY OF LEICESTER

DEPARTMENT OF BOTANY

Secondary product production by plant tissue cultures

A vacancy exists for a

RESEARCH TECHNICIAN

post in association with the above project. The post will be a Grade 3 limited tenure (two years) position on the following salary scale: £3,122 to £3,553. Candidates with appropriate qualifications and, if possible, experience in plant cell or tissue culture should apply to Professor H. Smith, Botany Department, University of Leicester, Leicester LE1 7RH. 3433(A)

RESEARCH SCIENTISTS BIOLOGICAL SCIENCES

Chicago, Illinois
USA Positions

The Life Sciences Division of IIT Research Institute, a not-for-profit Chicago, Illinois, USA based research organization has several staff positions for biologists. Requisite are PhD in physiology, toxicology, nutrition or experimental pathology. Preference will be given to candidates with post doctoral research experience and interest in long-term toxicity, carcinogenicity bioassay and carginogenesis studies in rodents. Opportunity is available to develop independent research projects in own field of expertise. Salary commensurate with experience and qualifications.

Please submit resumes including salary history to:

Ms. L. Amft
IIT
Research Institute
10 W. 35th St.
Chicago, Illinois, USA 60616

IITRI is an Equal Opportunity/Affirmative Action Employer and encourages applications from women and members of minority groups. W418(A)

UNIVERSITY OF BIRMINGHAM

Chemical Engineering

RESEARCH FELLOW IN THE MICROBIOLOGY OF FOULING

Applications are invited for a Researc Fellow in Biological fouling film formatic and removal, to be a member of a tea investigating the biological fouling of he exchange surfaces, for a period of tw years.

This involves the mechanisms of layir down, adhesion and removal of biologic fouling films on surfaces, and th microbiology of the deposits. The fello will also act as an advisor on micr biological aspects to the team and develo contacts with industry.

The person appointed is likely to be microbiologist or a biochemist biochemical engineer with a soun understanding of the fundamentals c microbiology.

The fellow will be based at AER Harwell, where the Heat Transfer an Fluid Flow Service is sponsoring an overa project on the fouling of heat exchang plant. The appointment is subject to th normal security arrangements presently a force at Harwell.

Salary will be in the RF1A range £4,33 to £7,521, plus superannuation. Maximur starting salary will be £5,777. Starting dat is as soon as can be arranged.

Further particulars are available fror the Assistant Registrar, Science an Engineering, University of Birminghan PO Box 363, Birmingham B15 2TT, t whom applications including ful curriculum vitae (3 copies) and namin three referees, should be sent by Friday 26th October, 1979.

Please quote reference NT9(r).

3418(A)

UNIVERISTY OF HEIDELBERG

INSTITUT FÜR THEORETISCHE ASTROPHYSIK

POSTDOCTORAL RESEARCH SCIENTIST

Applications are invited fror astrophysicists with interest in stella atmospheres, envelopes and interstella matter, radiation transport an hydrodynamics for a 2 or 3 year positio with possibility of extension to 5 years Minimum Salary DM 37.100.-p.a.

Applications with curriculum vita before 15 November 1979 to Prof. B Baschek, Institut Für Theoretisch Astrophysik, Im Neuenheimer Feld 294 D-6900 Heidelberg, West Germany.

W434(A

British Museum (Natural History) London

Head of Palynology Section

The British Museum (Natural History) is an institution for taxonomic research and an educational focal point for the public. The main aim of the research is to produce definitive accounts of the world's animals, plants and minerals as well as the preservation of an extensive collection of fossils. There are comprehensive library, computing and laboratory facilities.

The successful candidate will, as Head of the Palynology Section, be responsible for the curation and development of the research collections. The work will also involve assessing the effect of applying pollen characters to taxonomic problems, answering enquiries relating to palynology and supervising junior staff. There will be opportunities for limited involvement in general taxonomy and the possibility of field-work both in this country and abroad.

Candidates, normally aged under 32, should have a good honours degree or equivalent in Botany or closely related subject. At least 2 years' postgraduate experience in taxonomy is essential and practical experience of taxonomic palynology is highly desirable.

Appointment as Senior Scientific Officer £6,655 – £8,640 or Higher Scientific Officer £5,505 – £6,990 (to become £7,110 – £9,485 and £5,875 – £7,515 respectively from 1.1.80). Level of appointment and starting salary according to qualifications and experience. Promotion prospects. Non-contributory pension scheme.

For further information and an application form (to be returned by 9 November 1979) write to Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, or telephone Basingstoke (0256) 68551 (answering service operates outside office hours). *Please quote ref: SB/65/DK.*

3473(A)

UNIVERSITY OF BIRMINGHAM

DEPARTMENT OF GEOGRAPHY

LECTURESHIP

Applications are invited for the post of Lecturer in Geography. Candidates should have teaching and research interests in biogeography and soil studies. Salary will be on the scale £4,333 to £8,992, plus superannuation, according to age, experience and qualifications, but it is hoped to make an appointment towards the lower end of the scale.

Further particulars are available from the Assistant Registrar, Science and Engineering, University of Birmingham, PO Box 363, Birmingham B15 2TT, to whom applications (3 copies) including full curriculum vitae and naming three referees, should be sent by Friday 2nd November 1979. Please quote ref: NH1.

3462(A)

DEPARTMENT OF CHEMISTRY

APPOINTMENT IN BIOCHEMISTRY

Applications are invited for a tenure track appointment at the assistant professor rank, preferably with specialization in the area of macro-molecular biochemistry, starting September 1, 1980. The appointment is subject to budgetary approval. The successful applicant will be required to teach graduate and undergraduate courses in biochemistry and to establish a vigorous research program.

The current base of the assistant professor rank is $19,720 per annum.

Simon Fraser University is situated in Greater Vancouver, British Columbia.

Applications, including curriculum vitae, a concise statement of proposed research and the names of three referees, should be sent by December 31, 1979 to: Dr C H W Jones, Chairman, Department of Chemistry, Simon Fraser University, Burnaby, B.C. Canada V5A 1S6.

W426(A)

QUEEN MARY COLLEGE

(University of London)

DEPARTMENT OF CHEMISTRY

RESEARCH TECHNICIAN

Grade 6

required to take charge of organic technical services. Person with organising skill and broad practical knowledge of modern techniques and instrumentation required. HNC or equivalent sought. Salary scale £4,243 to £5,068 p.a. plus £780 p.a. London Weighting. 5 day week, 5 weeks annual leave, plus a week at Christmas and Easter including public holidays.

Letters only to Establishment Officer, C/T, Queen Mary College, Mile End Road, London E1 4NS, stating age, experience and qualifications. 3453(A)

Max Planck Institute Tübingen seeks a younger scientist with interests in the field of

CELL- AND DEVELOPMENTAL BIOLOGY OF THE NERVOUS SYSTEM AS

LEADER OF A RESEARCH GROUP

The successful applicant will have the opportunity to establish his own group and carry out an independent programme of research.

Applicants should already have achieved recognition in their field. The appointment is for a limited period (max. 5 years).

Funds for 1-2 research workers and two technical assistants are available, as well as appropriate materials and laboratory space.

In addition the Max Planck Institute has a further vacancy in the same field for a

RESEARCH SCIENTIST

Applications for these posts (closing date 30th November, 1979) should be sent to: The Geschäftsf. Direktor, Max-Plank-Institut für Virusforschung, Spemannstr. 35/II, D7400 Tübingen/W. Germany.

W428(A)

BIOCHEMIST

Topic: Parathormon dependent Calcium influence on lymphocyte functions

Application from suitably qualified candidates is invited for a research fellowship. Considerable experience in hormone-receptor interactions, ion transport and cellular biology is required. All modern technical equipment is available. Payment BAT II a. The appointment will be made for 3 years. Applications with curriculum vitae, recent publications and the name of two referees should be sent to: Professor Dr. R.D. Hesch, Calcium Metabolism Research Group, Dept. Endocrinology, Medizinische Hochschule Hannover, Karl-Wiechert Allee 9, Germany. W433(A)

MEDICAL RESEARCH COUNCIL OF NEW ZEALAND

ONE BIOCHEMIST ONE NEUROENDOCRINOLOGIST

(with either a neurophysiological or biochemical background)

For studies on the developmental endocrinology of the pig foetus. Both positions tenable for an intital period of 12 months, starting 1980.

Applications including a curriculum vitae, full details of qualifications and the names of two referees should be sent by Nov. 15, 1979 to: Forschungsbereich Endokrinologie und Neuroendokrinologie Institut für Tierzucht und Tierverhalten FAL Mariensee 3057 Neustadt 1 Federal Republic of Germany. W435(A)

M.R.C. CLINICAL RESEARCH CENTRE

(Northwick Park Hospital)
Watford Road, Harrow,
Middx HA1 3UJ.

Applications are invited for a

POST-DOCTORAL VIROLOGIST

to join the scientific staff on the connective tissue diseases study group and participate in basic studies concerning the effects of virus infections on the function of the immune system. This work is being applied to the search for viral aetiology of human auto-immune and connective diseases. This multi-disciplinary research group works within the division of rheumatology (head, Dr. B.M. Ansell), and in close conjunction with immunological medicine (head, Dr. G.L. Asherson) and communicable diseases (head, Dr. D.A.J. Tyrrell). The virologists will be expected to collaborate in the clinical research interests of this group but will also be free to develop more basic interests in collaboration with scientific staff of these divisions.

This is a limited term post and the appointment will be for a period of 4 or 5 years. Candidates should have a minimum of six years research experience since first degree.

Salary depending on age and experience within the range £5,591 to £8,452 plus £502 L.A. a year.

Potential applicants may discuss this appointment with Dr. A.M. Denman (tel. 01-864-5311 ext. 2235) before applying to Mr. A. Mariner for an application form, quoting ref. 131/1/3484, closing date 3rd November 1979. 3460(A)

New Product Planning for the 1980's

Pharmaceuticals: Ware, Herts.

An opportunity has arisen in our Medical and Development Division at Ware for an experienced graduate to join the team responsible for planning and co-ordinating the international development of new medicinal products, from the time of their identification to marketing. The department is also responsible for providing technical information on development projects to other Glaxo companies, both in the UK and overseas.
Applications are invited from graduates in an appropriate scientific discipline, aged around 30 with relevant experience, preferably gained within the pharmaceutical industry and ideally in the field of new product planning. Candidates must be able to co-operate and communicate effectively with all levels of staff in the various disciplines involved in pharmaceutical research and development.
We offer an attractive starting salary and an opportunity to participate in Glaxo Group profitability. Other benefits include non-contributory pension, and where appropriate, generous assistance with relocation to this attractive area of Hertfordshire.
Please write or telephone for an application form to:
Dr D. J. Humphreys, Personnel Officer, Glaxo Group Research Ltd., Ware, Herts SG12 0DJ. Tel: Ware 3232. 3452(A)

Glaxo Group Research Ltd.

UNIVERSITY OF LAUSANNE
DEPARTMENT OF PHARMACOLOGY
RESEARCH ASSISTANT

Applications are invited for this post available from 1 November 1979 for two years with possibility of renewal. Candidates (M.Sc., Ph.D, or MD) should be familiar with biochemical techniques, with experience in purification of proteins (including affinity chromatography) and production of antibodies. The successful candidate will work within our hypertension research group. Knowledge of French would be an advantage but is not a requirement.

Salary within range of Sw. Frs. 27'700.--to Sw. Frs. 47'607.-- p.a.

Applicants should send a curriculum vitae and names of three referees to: Prof. G. Peters, M.D., Institut de pharmacologie de l'Université de Lausanne, Rue du Bugnon 21, CH-1011, *Lausanne*, (Switzerland) W405(A)

POLYMER SCIENTIST

Hellermann Electric is a member of the Bowthorpe Holdings Group of International Companies supplying components and accessories to the electrical and electronic industries throughout the world. Our new factory at Plymouth specialises in the supply of cable stripping, marking, insulating and securing systems. We also specialise in heat-shrinkable materials and supply equipment and accessories of all kinds.

We now require a Polymer Scientist to join a small team developing heat shrinkable plastic products. Applicants should hold a PhD and have a minimum of 2 years post-doctoral experience preferably in processing elastomers. Scientific excellence and a willingness and ability to work in a variety of fields, some of which may be outside their current speciality, is of more importance than their specific training.

Salary negotiable with good Company benefits including re-location expenses.

Please apply to:
R. MANSON
PERSONNEL MANAGER
HELLERMAN ELECTRIC
Pennycross Close, Plymouth, PL2 3NX
(0752) 701261
3458(A)

OXFORD UNIVERSITY
Sir William Dunn
School of Pathology
PLASMA MEMBRANE BIOCHEMIST

Applications are invited for a post-doctoral research position, funded by a grant from the Medical Research Council. The research concerns the characterisation of macrophage antigens detected by monoclonal antibodies. Experience in membrane biochemistry and in immunochemistry is desirable. The appointment, starting as soon as possible after 1st October 1980, is likely to be for 3 years. The salary will be at an appropriate point on the University 1A scale. £4,333 to £7,145.

Applications, including *curriculum vitae* and the names and addresses of 2 referees, should be sent to Dr S. Gordon, Sir William Dunn School of Pathology, South Parks Road, Oxford OX1 3RE. 3369(A)

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London
3 Dyers Buildings
London EC1N 2NR
01-831 6901

New York
Suite 832
50 Rockefeller Plaza
New York, NY 10020
(212) 765-5758

UNIVERSITY OF LEICESTER
DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS
POSTDOCTORAL RESEARCH ASSOCIATE

Applications are invited for a postdoctoral research associateship to work on the characteristics and regulation of catecholamine receptors in the central nervous system. Experience in receptor labelling techniques and cyclic nucleotide metabolism would be advantageous but not essential. Applicants should be recent PhD graduates or have up to three years postdoctoral experience. The post is for two years and the salary will be in the Range 1A (£4,333 to £7,521) depending upon age and experience.

Applications giving details of qualifications, research experience and the names of two academic referees should be sent to Dr S.R. Nahorski, Department of Pharmacology and Therapeutics, School of Medicine, Medical Sciences Building, University of Leicester, University Road, Leicester. LE1 7RH (Tel: 0533 551234, Ext. 361). 3358(A)

COMMONWEALTH AGRICULTURAL BUREAUX
COMMONWEALTH MYCOLOGICAL INSTITUTE
Kew, Surrey
MYCOLOGIST

The Commonwealth Mycological Institut has a vacancy for a mycologist to undertak the identification of microfungi. There ar opportunities for taxonomic research an the successful applicant will be expected t become an authority on a selected group o fungi.

Qualifications: A good honours degre preferably with postgraduate experience o the systematics of microfungi.

Starting salary: According to age qualifications and experience, in the scal £3,289 to £6,211, plus London Weightin £325. Age normally under 27.

Applications by 30 November to th Executive Director, Commonwealt Agricultural Bureaux, Farnham House Farnham Royal, Slogh SL2 3BN, fro whom application forms and particular can be obtained. 3470(A)

NATIONAL INSTITUTE FOR RESEARCH IN DAIRYING
(University of Reading)
CHEMISTRY
DEPARTMENT

A graduate is required to work for thre years on the effect of heat on the protein and enzymes of human milk. The wor involves undertaking a detailed study of th kinetics of their heat denaturation an relating their results to the effect o pasteurisation treatments on human milk

Qualifications: First or Upper Secon Class Honours degree in biochemistry o food science. An opportunity exists for suitable candidate to register for a highe degree.

Appointment, for a limited period o only three years, will be as Scientifi Officer; salary scale £3,289 to £5,08 (£3,591 to £5,486 from 1.1.80). Non contributory superannuation.

Application forms obtainable from th Secretary, NIRD, Shinfield, Reading RG 9AT. Quote reference: 79/32. 3350(A)

UNIVERSITY OF NEWCASTLE UPON TYNE

BIOCHEMISTRY RESEARCH DEMONSTRATORSHIP

Applications are invited for the post of

DEMONSTRATOR

in the Department of Biochemistry from candidates who possess, or have submitted for, a PhD in Biochemistry, for a period of up to three years, tenable from 1st January, 1980. Research projects currently in progress include membrane transport of nucleic acid precursors (Professor K. Burton), acquisition and modification of enzyme conformation, and immunochemistry of monocyte surface (Professor R.H. Paine), DNA repair and genetic recombination (Dr P.T. Emmerson), triglyceride and lipoprotein metabolism (Dr M.P. Rogers), enzymology of resistance to antibiotics (Dr R. Virden), lactose transport and bioenergetics (Dr I.C. West) and hormonal control of enzyme activity (Dr S.J. Yeaman). Duties will also include teaching (approximately 90 hours per year) by lectures, tutorials and practical classes.

Salary will be at an appropriate point on the Grade 1B (Bar) scale £3,775 to £5,488 per annum according to age, qualifications and experience. Membership of the appropriate University superannuation scheme will be required.

Further particulars may be obtained from the Senior Assistant Registrar (F.P.) The University, 6 Kensington Terrace, Newcastle upon Tyne, NE1 7RU, with whom applications (2 copies), giving the names and addresses of three referees, should be lodged as soon as possible.

3376(A)

THE UNIVERSITY OF MANCHESTER

DEPARTMENT OF CHEMISTRY

POST-DOCTORAL RESEARCH FELLOW

Applications are invited for a Post-Doctoral Research Fellow to work in conjunction with Dr C D Garner, Dr F Knowles (of Leeds University) and Dr J Blackburn (UMIST) on a study of the detailed molecular environment of copper centres in enzymes using X-Ray Absorption Spectroscopy (EXAFS). Experience in the isolation of biological materials would be particularly advantageous and a willingness to participate in the measurement and interpretation of the spectroscopic data will be welcomed. The appointment will be for one year initially, from October 1st 1979 or as soon as possible thereafter, and is renewable for a further two years. Salary will be in the range £4,333 to £4,910.

Applications, with a curriculum vitae and the names of two referees, should be sent to Dr C.D. Garner, Department of Chemistry, The University, Manchester M13 9PL, as soon as possible. 3451(A)

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New York, NY 10020
(212) 765-5758

PRODUCT REGISTRATION OFFICER

Pfizer Central Research, which this year was granted a Queen's Award for technological achievement, is part of a multinational organisation engaged in the research, development, manufacture and marketing of a wide range of human medicinal and animal health products.

Our Product Registration Department which is responsible for advising on the requirements of regulatory authorities throughout Europe, and for obtaining approval to undertake clinical studies on field trials, and subsequently, marketing of new human and veterinary drugs arising from our research laboratories both in the U.K. and U.S.A. now needs an additional Product Registration Officer.

The person appointed to this post will probably be in his/her late twenties or early thirties, have a degree in a science-based discipline, with ideally a post-graduate qualification and several years experience in registration work of both human and veterinary products. He/she will participate in all stages of the progression of a number of development candidates and compile the documentation necessary for regulatory submissions. In time extensive interaction with regulatory agencies and other Pfizer personnel in Europe will be required thus providing considerable scope for individual responsibility.

The starting salary will reflect qualifications and experience and in addition to excellent career prospects there is good bonus potential. Pension, death benefit and salary continuance schemes are in operation and there is generous assistance with re-location expenses to a pleasant coastal/rural area of East Kent.

Flexible hours are worked and the social/recreational facilities are first class.

3457(A)

Pfizer

Applications giving brief details of age, qualifications and experience should be sent to:

D.W. Sells, Personnel Manager, Pfizer Central Research, Sandwich, Kent.

1979

COMMONWEALTH AGRICULTURAL BUREAUX

Vacancy for

SCIENTIFIC INFORMATION OFFICER

at the

COMMONWEALTH BUREAU OF ANIMAL HEALTH

Central Veterinary Laboratory, New Haw, Weybridge, Surrey.

General information. The work of the Bureau is to collect and make available through its publications *The Veterinary Bulletin* and *Index Veterinarius* and by magnetic tape services, information relating to all aspects of veterinary science.

Main duties. The duties include searching scientific periodicals, indexing articles, preparing English-language abstracts for the monthly journal *The Veterinary Bulletin*, editorial work, dealing with enquiries and preparing reviews and bibliographies.

Qualifications. A qualification in information science or an appropriate biological science is required, plus a knowledge of European and Slavonic languages or a willingness to learn languages.

Salary. In the scale £3,289 to £6,211 per annum plus an Outer London Weighting allowance of £325 per annum, plus a compensatory allowance (taxable but not superannuable) of 3.3% to offset personal contribution to Superannuation Scheme — part-time appointment *pro rata*. Starting salary according to qualifications, experience and age.

Application forms and full particulars from the Executive Director, Commonwealth Agricultural Bureaux, Farnham House, Farnham Royal, Slough SL2 3BN, to whom completed applications (in duplicate) should be sent by 30 November 1979. 3474(A)

MICROBIOLOGY

A regular tenure track position as an Assistant or Associate Professor is available commencing as soon as possible for an M.D. or a PhD with a minimum of two years postdoctoral training in microbial physiology or in infectious disease.

Send curriculum vitae and names of three referees to: Dr David Eidinger, Head, Department of Microbiology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 0W0. Closing date for receipt of applications is November 30, 1979. W431(A)

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE

(University of London)

DEPARTMENT OF PHARMACOLOGY

A JUNIOR TECHNICIAN

is required to assist with teaching and research work in the Department which is situated in the Clinical Sciences Building at the Royal Free Hospital in Pond Street, Hamstead. Experience in electron microscopy is essential.

Salary according to age and qualifications on Whitley Council Scales.

Application forms are available from the School Secretary, R.F.H.S.M., 8 Hunter Street, London, WC1N 1BP or telephone 01-837 5385 Ext. 10. Closing date: 26 October, 1979. 3464(A)

NEWCASTLE AREA HEALTH AUTHORITY (TEACHING)

NEWCASTLE GENERAL HOSPITAL MEDICAL LABORATORY

SCIENTIFIC OFFICER

required in Muscular Dystrophy Group Laboratories, Regional Neurological Centre. The successful applicant will assist with development of tissue culture techniques for study of muscle and nervous tissues as part of a research programme which involves several scientific disciplines. Experience of tissue culture and sterile techniques and a knowledge of electron microscopy an advantage. Candidates must be registered.

Further information from Mr J. J. Fulthorpe, Senior Chief MLSO, Tel: 0632-738811 extn 573.

Applications, with names and addresses of two professional referees, to Western Sector Administrator, Newcastle General Hospital, Newcastle upon Tyne NE4 6BE, by 19th October, 1979. 3424(A)

The Department of Geology and Geophysics of the State University of Utrecht has a temporary position (2 years) available for a

MINERALOGIST

He/She will work, in collaboration with a team of two other mineralogists, within the section of Petrology/ Mineralogy/Crystallography/Geochemistry/Pedology.

- take part in mineralogical courses (systematic mineralogy, mineralogical analytical methods). He/She will be mainly responsible for the organisation of new course on applied mineralogy.
- set up new research in applied mineralogy (mineralogical problems in industry, as for example the quality control of industrial minerals and processes for improvement of the properties of synthetic minerals).

Applicants must be mineralogists or chemist/chemical engineers with experience in applied mineralogy.

Salary depending on age and experience.

Information can be obtained from Prof. Dr. P. Hartman, Geological Institute, Garenmarkt 1b, Leiden, tel.: 071-148333 ext. 7337.

Candidates are requested to submit their application within three weeks to the Personnel Department, Geological Institute, Oude Gracht 320, Utrecht, The Netherlands. W424(A)

Max-Planck-Institut für Radioastronomie
Sonderforschungsbereich Radioastronomie

RESEARCH SCIENTIST or ENGINEER
MILLIMETER WAVELENGTH TECHNOLOGY

The MPIfR has a strong involvement in instrumental developments for radio astronomy in the millimeter and sub-millimeter wavelength range. It participates heavily in the construction and instrumentation of a 30-m diameter mm-telescope for the new French-German Institute for Radio Astronomy in the Millimeter Range (IRAM). Developments in the sub-millimeter region are underway in the fields of telescope and receiver design.

A physicist/electronics engineer is sought for the development of circuits in the frequency range 100-400 GHz and the integration of these to low-noise radio astronomy receivers. Knowledge and experience in the field of micro- or millimeter waves is required.

Momentarily the position is financed by the Deutsche Forschungsgemeinschaft and is time limited to end 1981. It is expected to continue the position after that date as a staff position at MPIfR. Salary according to the German government employees rules (BAT). Send application with résumé of education, experience and special interests

Max-Planck-Institut für Radioastronomie
Auf dem Hügel 69
D-5300 Bonn 1
Germany

W422(A)

Surrey Area Health Authority
West Surrey/North East HAMPSHIRE HEALTH DISTRICT
Frimley Park Hospital
Basic Grade Biochemist

Applications required from suitably qualified persons to work in the Department of Chemical Pathology, Frimley Park Hospital. The Department serves a large District General Hospital and has strong links with St Lukes Hospital, Guildford and with the Department of Biochemistry, University of Surrey. The Laboratory is well equipped and provides a general analytical service. This is a busy Department offering a wide range of investigations, and the successful applicant will contribute to the routine commitment of the Department and will be expected to participate in research and development work which is presently underway in the field of Radioimmunoassay.

Salary scale: £3,627 to £5,733 inclusive of Outer London Weighting.

Further information available from Dr W I Alston, telephone Camberley 62121 Ext 4129.

Application forms are available from the Personnel Department, Frimley Park Hospital, Portsmouth Road, Frimley, Camberley, Surrey, telephone Camberley 62121 Ext 4005. 3417(A)

A senior level position is open for a
BIOCHEMICAL PHARMACOLOGIST
DEPARTMENT OF BIOCHEMISTRY

in affiliation with the Oncology Research Center. The candidate would be responsible for a program to develop anti-tumor agents for eventual use in drug trials. A strong emphasis in the mechanism of action of potential drugs is essential.

Interested candidates should contact Dr. M. Waite, Department of Biochemistry, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem North Carolina 27103. Telephone (919) 727 4373. Affirmative Action Equal Opportunity Employer. W430(A)

ATHROFA GOGLEDD-DD CYMRU
THE NORTH E WALES INSTITUTE OF HIGHER EDUCATION

Kelsterton College,
Connah's Quay,
Deeside, Clwyd

SRC CASE STUDENTSHIPS

Applications are invited for a SRC CASE Studentship tenable immediately for work on the following project: "Radio-immunoassay of polyamines", in collaboration with Ortho Diagnostics, Ltd. Supervisors: Drs. J. C. Allen and C. J. Smith.

The co-operating body will supplement the SRC Studentship by £250 p.a. Applicants should possess or expect to obtain 1st or upper 2nd class Honours, and should write as soon as possible, with curriculum vitae, to the Institute Registrar, North E Wales Institute, Kelsterton College, Connah's Quay, Deeside, Clwyd CH5 4BR. 3395(F)

KING'S COLLEGE LONDON
ELECTRONICS TECHNICIAN

For interesting work in busy Physics Research Department including building, repair and maintenance of equipment. Experience in integrated circuits and digital electronics desirable. Good conditions. Five weeks annual holiday. Superannuation scheme. Interest-free loans for annual rail season tickets. Salary on scale £4,580 p.a. to £4,844 p.a. inclusive (under review).

Apply in writing with full details to: The Head Clerk (Ref: 221721/N), King's College London, Strand, WC2R 2LS. 3471(A)

UNIVERSITY OF CAMBRIDGE
DEPARTMENT OF PHYSICS

A Royal Society sponsored
POSTDOCTORAL RESEARCH ASSOCIATE

is required as soon as possible to join a ne group in the Cavendish Laboratory t undertake the development of ne instrumentation and apparatus for Surfac Physics research. The work is expected t include initially the design of electron optical instrumentation such as electro spectrometers, electron and photo detectors, and imaging devices, togethe with related electronics and computin techniques.

Experience and a high level of expertis in U.H.V. engineering design, cryogenic and in electronics is desirable. A degree o equivalent qualification is preferre although proven abilities in the abov techniques are the prime consideration Salary on a scale to £6,932 p.a. according t age and experience.

Three copies of applications including full c.v. and names of referees should b sent to Mr J. Deakin, Secretary of th Department of Physics, Cavendis Laboratory, Madingley Road, Cambridg CB3 0HE before 31 October. 3407(A)

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Director of Biology

G. D. Searle is a leading international pharmaceutical company with its main U.K. headquarters and research facilities at High Wycombe in Buckinghamshire.

Applications are invited for the position of Director of Biology from persons with qualifications and research experience in a Biological Science, preferably Pharmacology. This position reports to the Director of Research.

Responsibilities include directing the efforts of a number of groups of biological scientists working principally in the area of infectious diseases, immunology and tumour biology. In addition to drug testing programmes, studies on the mechanism of action of therapeutic agents and on the basic biology of the diseases are undertaken. The successful applicant must therefore have a broad view of biology and medicine, whilst being able to direct in depth studies in particular areas. The responsibilities also include management of the animal facilities, so direct experience is essential. Experience in supervision and administration of other licencees will be advantageous.

The groups within the Research Department are organised to encourage an interdisciplinary approach to most studies and past experience of this aspect within the Pharmaceutical Industry will be a strong recommendation. In addition evidence of managerial experience will be required.

This is a senior position, carrying a place on the Management Group of the Research Department, and the salary, which is negotiable, will reflect this seniority.

The benefits accompanying this post include: company car, relocation assistance, life insurance, contributory pension fund, private medical scheme, and sports and social club.

Applications should be addressed to Mr G. Jenkins, Personnel Manager, Searle Research & Development, G. D. Searle & Co. Ltd., Lane End Road, High Wycombe, Bucks (Tel. High Wycombe 21124, ext. 3374).

SEARLE

3396(A)

UNIVERSITY OF ST. ANDREWS

DEPARTMENT OF PSYCHOLOGY

RESEARCH ASSISTANT INFANT DEVELOPMENT

pplications are invited for a post of esearch Assistant to work for one year on e final phase of a study of mother/infant teraction conducted by Dr. A. Whiten. It hoped to appoint a Postdoctoral esearch Assistant but candidates without PhD will also be considered. Candidates ould be able to commence work as soon s possible and not later than November.

Starting salary at appropriate point ithin range £3,775 to £4,910 per annum nder review) according to qualifications d experience, plus FSSU/USS.

Applications with the names of 3 referees ould be sent as soon as possible to Dr A. hiten, Department of Psychology, The niversity, St. Andrews, Fife, from whom rther particulars may be obtained.

3450(A)

NIVERSITY DEPARTMENT OF CLINICAL PHARMACOLOGY

MEDICAL RESEARCH COUNCIL UNIT OF CLINICAL PHARMACOLOGY

Radcliffe Infirmary
Oxford OX2 6HE

TECHNICIAN

plications are invited for this post to sist with research into the effect of drugs human platelet receptors. Familiarity th biochemical techniques would be an vantage.

Applicants should have HNC, degree or uivalent qualifications. Salary will be thin the range £3,768 to £5,391, pending on age and experience.

Applications with the names of two erees should be sent to The Chief chnician, MRC Clinical Pharmacology nit, Radcliffe Infirmary, Oxford OX2 E by 20th October 1979.

3461(A)

CARDIOTHORACIC INSTITUTE

DEPARTMENT OF MEDICINE

UNIVERSITY OF LONDON

MEDICAL LABORATORY SCIENTIFIC OFFICER OR RESEARCH ASSISTANT

required. Experience in handling lymphocytes is essential. Work will mainly involve the laboratory investigations of immunological responses in patients with Lung Cancer.

Applications stating Ref: 01/79 and giving age, experience and the names of two referees to Mr R.A. Perkins, Secretary, Cardiothoracic Institute, Fulham Road, London, SW3 6HP.

3435(A)

ASSISTANTSHIPS

UNIVERSITY OF LEICESTER

DEPARTMENT OF BOTANY

Postdoctoral/postgraduate research assistantship in secondary product production by plant tissue cultures

Two posts are available for research on the above project to be held either at postdoctoral or postgraduate research assistant level. In the case of a successful applicant's being appointed at the postdoctoral level the appointment will be for two years only. In the case of a candidate's being appointed at postgraduate level funds will be available for three years and registration for higher degrees will be possible.

Further information on the project and related conditions may be obtained from Professor H. Smith, Botany department, University of Leicester, Leicester LE1 7RH.

3432(P)

FELLOWSHIPS

MONASH UNIVERSITY

MONASH POST-DOCTORAL FELLOWSHIPS

Applications are invited for up to 4 positions in any department of the University. Posts are annually renewable from 1st January 1980 for up to 2 years. Fellows will be expected to collaborate in their research with a member of the University staff. Appointment in the Junior Research Fellow range ($A14,041 to 15,676) but Research Fellow appointment ($A16,291 to 21,401) may be considered in exceptional circumstance.

For further information please write to the Chairman of the relevant department through Mr J. D. Butchart, Academic Registrar.

Applications including Ref. No 90162, curriculum vitae and 2 referees to the Academic Registrar, Monash University, Clayton, Victoria, 3168, Australia by 30 November 1979.

3454(E)

UNIVERSITY OF MARBURG

Germany

POSTDOCTORAL FELLOWSHIP in Molecular Biology

To study the structure and expression of steroid regulated genes in mammals. Experience in modern nucleic acid biochemistry requested.

The position is open starting January 1980 for three years, with a salary of about DM 40000 p.a. Candidates should send c.v. and the names of three referees to: Prof. Dr M. Beato, Institut für Physiologische Chemie, Deutschhausstr. 2, D-3550 Marburg, F.R.G.

W419(E)

UNIVERSITY OF NOTTINGHAM

SCHOOL OF AGRICULTURE

Department of Physiology & Environmental Studies

POST-DOCTORAL FELLOWSHIPS IN REPRODUCTIVE PHYSIOLOGY

Two post-doctoral fellowships are available for 15 months to assist in established research projects in bovine reproduction concerning a) hormonal control of ovulation and b) embryonic mortality.

Applications are invited from agricultural science, biology or veterinary graduates. Experience in handling large mammals and using radioimmunoassays an advantage. Qualified predoctoral candidates will also be considered. Salary £4,333 to £4,910 p.a. (Pre-Doctoral £3,775 to £4,333).

Application forms and further details can be obtained from The Secretary, University of Nottingham, School of Agriculture, Sutton Bonington, Loughborough, Leics.

3465(E)

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are invited for appointment ove-mentioned position in the ent of Physical and Inorganic stry (Ref: 5). Two year temporary ointment to commence 1 January 1980, as soon as possible thereafter. Candidates should be PhD graduates in the field of electrochemistry, preferably with a background or strong interest in biochemistry. Research is to investigate in collaboration with Associate Professor Ritchie the effect of enzymes on electrochemical reactions particularly oxygen reduction. Well equipped electrochemical laboratory with the usual workshop facilities. Further information available from the Acting Staffing Officer in the University, or from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. Salary range: $A16,291 to $A17,751 per annum (approximate exchange rate: $A1.00 = $US1.12 = £0.50); assistance towards fares and removal expenses will be provided.

Applications in duplicate stating full personal particulars, qualifications and experience and date of availability to reach the Acting Staffing Officer, The University of Western Australia, Nedlands, 6009, Western Australia by 9 November, 1979. Candidates should request three referees to write immediately to the Acting Staffing Officer. 3430(E)

-Unique Opportunity-
STANFORD UNIVERSITY
NSF POSTDOCTORAL FELLOWSHIP in MOLECULAR BIOLOGY and COMPUTER SCIENCE
$14,000 per annum

Applicants should be familiar with the techniques of modern molecular biology and be interested in entering the field of computer science. Previous programming experience is useful but not essential. Responsibilities will include development and maintenance of a knowledge base of current laboratory techniques for use with an advanced model of automated experimental planning. One goal of this program is to train an individual for a career in computer science as applied to molecular biology and genetics. While the fellowship will be awarded through the Department of Computer Science, the fellow will interact regularly with research groups in the Departments of Biochemistry and Medicine.

Send resume and the names of three references to either:
Dr Douglas Brutlag, Department of Biochemistry or Dr L.H. Kedes, Department of Medicine, Stanford University, School of Medicine, Stanford, California 94305. Equal opportunity through affirmative action. W383(E)

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE
(University of London)
JUNIOR RESEARCH FELLOWSHIP

Applications are invited for a Junior Research Fellowship £2,250 p.a. plus approved fees), tenable for 3 years in the Department of Biochemistry and Chemistry for work on erythrocyte membranes in single cell disease using fluorescence, gel electrophoresis and scanning electron microscopy, leading to a PhD degree of London University.

Applications, with curriculum vitae and names of two referees, to be sent as soon as possible to Professor J.A. Lucy, Royal Free Hospital School of Medicine, 8 Hunter Street, London WC1N 1BP. 3466(E)

UNIVERSITY OF NOTTINGHAM MEDICAL SCHOOL
POST-DOCTORAL FELLOWSHIP IN MUSCLE BIOCHEMISTRY

A new group has been established in the Department of Physiology and Pharmacology under the leadership of Dr. C.T.M. Davies (M.R.C.) to investigate the physiology and biochemistry of human muscle in the elderly.

Postgraduates with appropriate experience of biochemistry interested in this fellowship should write in the first instance to Professor P.H. Fentem, Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham, NG7 2UH, enclosing a curriculum vitae and the names of two referees. 3449(E)

STUDENTSHIPS

UNIVERSITY OF LIVERPOOL
DEPARTMENT OF DENTAL SCIENCES
RESEARCH STUDENTSHIP

Applications are invited from honours graduates in either chemistry, biology or biochemistry to join a group working on the physical principles involved in the interactions of biological macromolecules that lead to the formation of dental plaque. The programme is concerned with the isolation, identification and properties of those salivary and bacterial components that lead to their aggregation and to their adhesion to the tooth surfaces. The studentship will be available for 3 years and the successful candidate will be expected to read for a higher degree.

Applications, together with the names of two referees, should be received as soon as possible by The Registrar, The University, PO Box 147, Liverpool, L69 3BX. Quote Ref. RV/799/N. 3416(F)

The University of Adelaide
Department of Agronomy
Applications are invited for a
Research Studentship

to support research for PhD with Dr J H Silsbury of the Waite Agricultural Research Institute on an Australian Wheat Industry Research Council project concerned with Nitrogen accumulation by field grown legumes. Tenure of the studentship depends on continuing support from the Australian Wheat Industry Research Council but at least three years support is expected. The stipend payable is $A4,200 p.a. Applicants should be graduates with a good honour's degree in a science subject and an interest in one or more of the following would be an advantage: crop physiology, CO_2 assimilation and nitrogen fixation.

Applications with a curriculum vitae and the names and addresses of two academic referees should be sent to the Academic Registrar, The University of Adelaide, GPO Box 498, Adelaide South Australia, Australia 5001. Closing date 31 October, 1978. 3420(F)

ANNOUNCEMENTS

NATO ADVANCED STUDY INSTITUTE
Exploration of the Polar Upper Atmosphere
May 5 to 16, 1980
Lillehammer Turisthotell, Norway

For information please contact: Professor A. Egeland, Institute of Physics, University of Oslo PO Box 1038 Blindern, Oslo 3, Norway.

Deadline for application February 1, 1980. W429(G)

LECTURES

LEUKAEMIA RESEARCH FUND

1979 Guest Lecture
will be delivered on
Tuesday 23rd October 1979 at 5.30 p.m.
at the Institute of Child Health
Guilford Street, London WC1
by **Professor Emil J. Freireich, MD**
of the Texas Medical Center, Houston, U.S.A.
subject: **"The Criteria for Curability for Adult Acute Leukaemia"**
Admission is free by ticket from:
Leukaemia Research Fund,
43 Great Ormond Street,
London WC1N 3JJ Tel: 01-405 0101

3442(K)

COURSES & CONFERENCES

UNIVERSITY COLLEGE DUBLIN
UNESCO — EEC
POST-GRADUATE COURSE IN TECHNIQUES IN ADVANCED INORGANIC CHEMISTRY

University College, Dublin in collaboration with the Irish National Commission for UNESCO, the Irish Department of Foreign Affairs and the Commission of the European Communities, Directorate-General for Development, Training Division wish to announce a Post-Graduate Course in Techniques in Advanced Inorganic Chemistry specially designed for students from developing countries who are presently employed in tertiary teaching or research institutes.

The Course will run from Tuesday, 5th August, 1980 until October 31st, 1980 and will commence with an intensive formal course of five weeks, involving lectures and laboratory work which will cover a wide range of topics such as advanced valence theory, molecular orbital methods, organometallic chemistry and diverse structural techniques (X-ray diffraction, electron diffraction, N.M.R. spectroscopy including ^{13}C N.M.R., magnetochemistry, E.S.R., IR.). The formal course will be followed by research work on a project in one of the above fields connected with the research interests of the present Inorganic Staff of the Department, which embrace Organometallic Chemistry, Coordination Chemistry and Theoretical Inorganic Chemistry.

Students applying for this course will be expected to have a Master's Degree. The language of instruction will be English.

Fellowships

A number of Irish Government sponsored scholarships are available to cover living costs. UNESCO will provide some fellowships to cover travel costs. The EEC will also provide fellowships to participants from ACP countries.

Applications

Application giving full details of the candidate's academic background, experience and names of referees should be made through the UNESCO National Commission of their respective country. Participants from ACP countries may apply to their own government for an EEC fellowship covering travel expenses to Ireland and course fees, including accommodation.

Completed applications should be sent to the Secretary, Chemistry Department, University College, Dublin, *before 31st December, 1979.*

3443(D)

INTERNATIONAL CONFERENCE ON HUMAN HERPESVIRUSES

Emory University March 17-21, 1980

This meeting is aimed at developing an overall view of all human Herpesviruses (Herpes Simplex 1 and 2, Varicella-zoster, Cytomegalovirus, and Epstein-Barr virus) from the molecular to the epidemiological and from diagnosis to treatment, by providing a forum for interchange on recent basic and clinical information.

For further information contact: Dr. André Nahmias, Emory University, 69 Butler Street, S.E., Atlanta, Georgia, 30303 or call 404-588-4997. W432(C)

Typesetting: J. W. Arrowsmith Ltd, Bristol, and Outline Graphics Ltd, 61 Fleet St, London EC4. Printing: Web Offset by The Chesham Press Ltd, Chesham, Bucks, in association with Godfrey Lang

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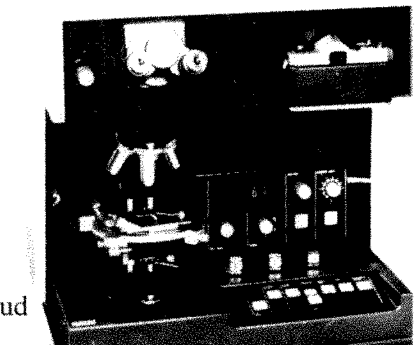

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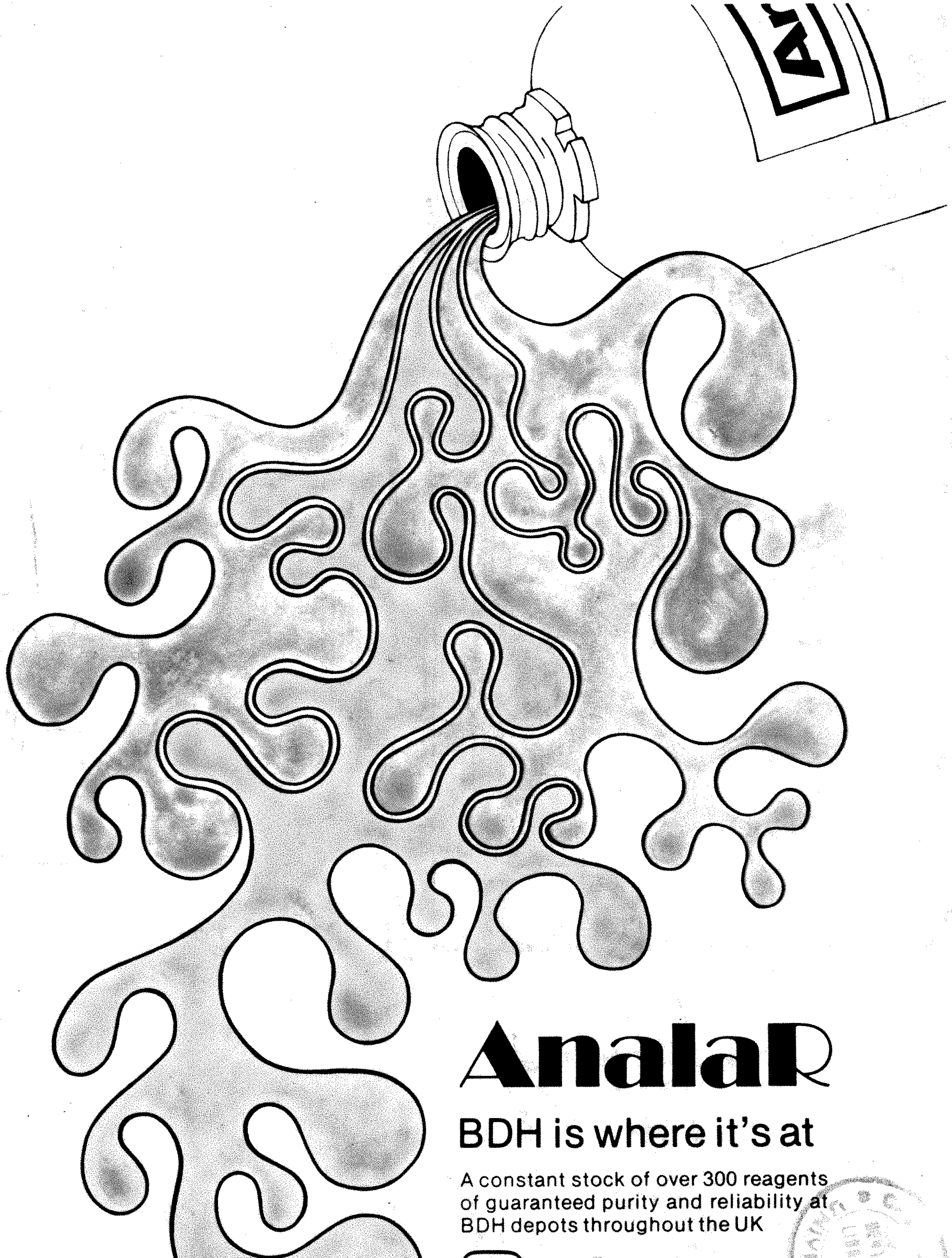

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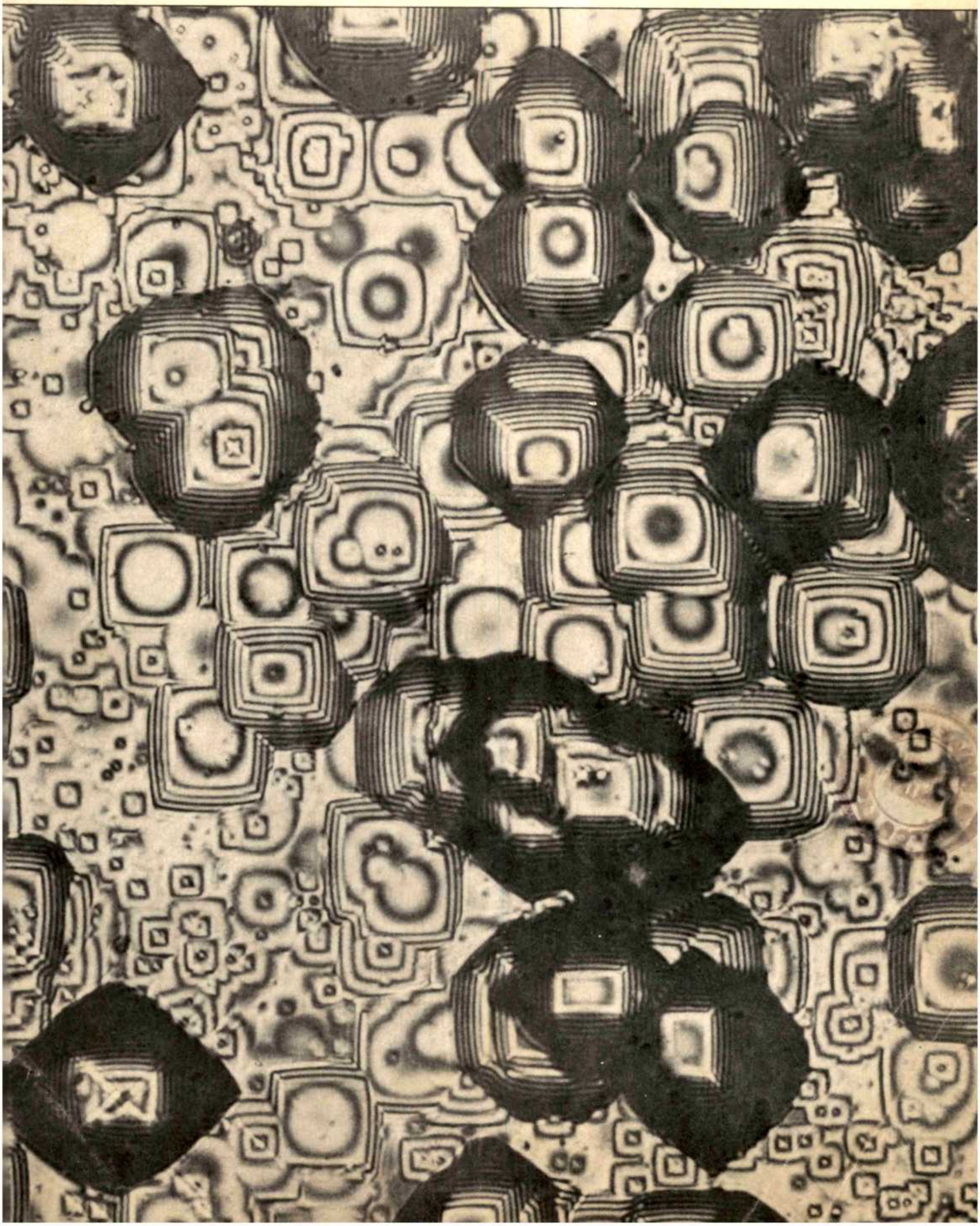

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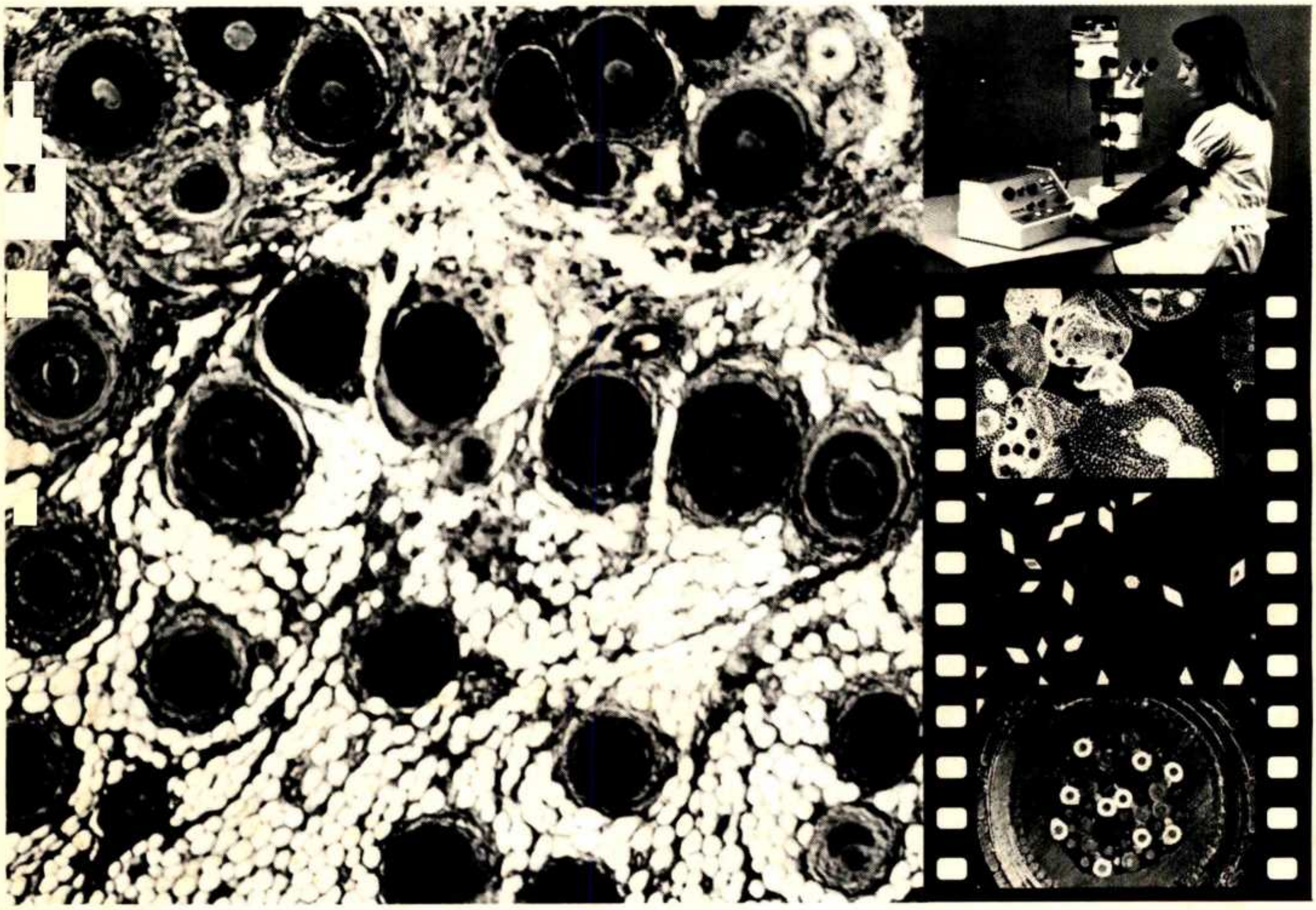

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Telegrams: Phusis London WC2R 3LF

Editor: David Davies
Deputy Editor: Peter Newmark

Editorial Staff
Philip Campbell Sara Nash
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Publishing Director: Christopher Paterson

New York
175 Fifth Avenue, New York, NY 10010
Telephone: (212) 777 5858 Telex: 668497
Robert Ubell (American Publisher)
and Sheila Kane

Washington News Bureau
711 National Press Building, DC 20045
Telephone: (202) 737 2355 Telex: 64280
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London Advertisement Manager:
Richard Webb
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Cover picture

Interferometry of (100) face (0.33 × 0.37mm) of synthetic cubo-octahedral diamond etched (statically) in potassium nitrate at 920 K for 1.5 hours. John Walker reviews *The Properties of Diamond* on page 611.

(From Tolansky, Miller & Punglia, *Phil.Mag.*, 1972.)

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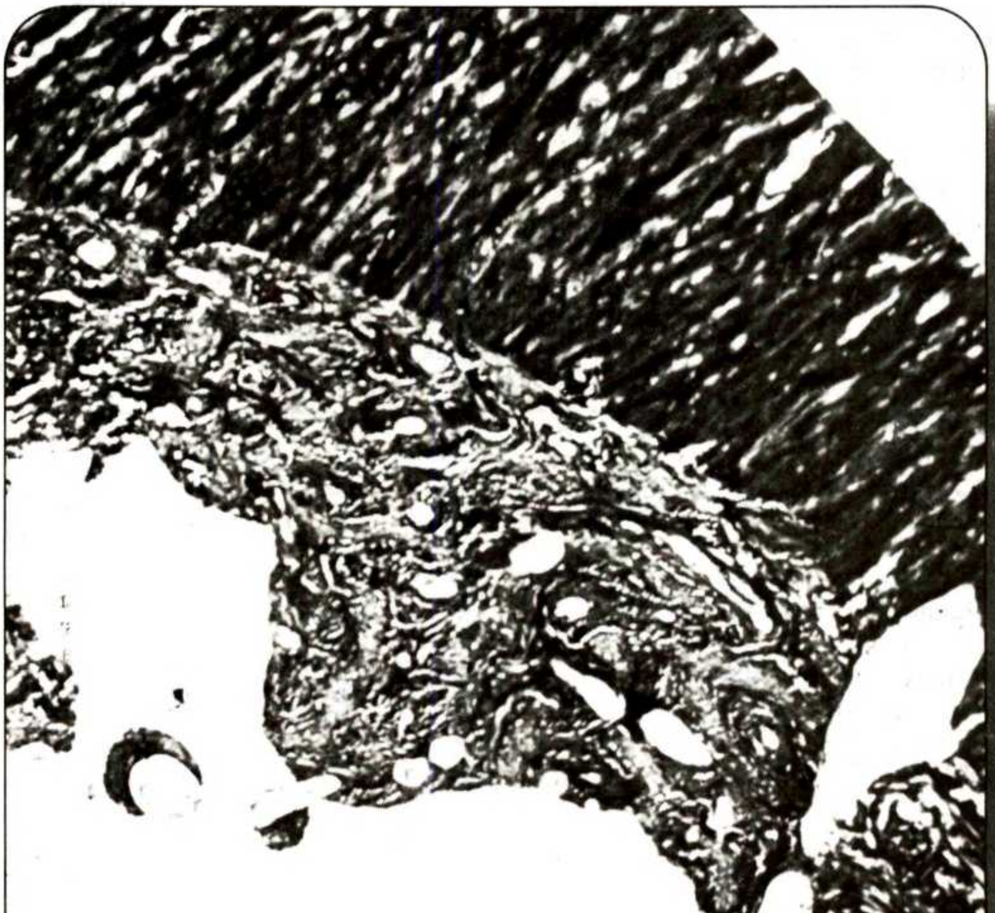
This month's new products feature focuses on radioimmunoassay and electrophoresis, and is found on p.xxi. Further information about these products can be obtained from the manufacturers, using our reader enquiry card service.

Helping nature to help itself

Internal fixation, or osteosynthesis, of bone by means of metal pins, screws, plates or wires, is often necessary to aid restoration of continuity and stability during healing of a fracture. However, the problems of rejection or encapsulation of such prostheses still remain.

One possible alternative to internal fixation is the use of a new bone structure developed jointly by the Dentistry Sub-Faculty of the Free University of Amsterdam and Philips Research Laboratories.

Tri-calcium phosphate powder, with a particle diameter of 2-3 microns, together with water and an oxygen-producing additive, was sintered at 1100-1400°C to produce a porous bio-degradable ceramic possessing the same compression strength as bony tissue. The procedure of treating the phosphate powder slurry and the subsequent sintering was chosen such that the porosity, or percentage of pores that de-



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termine tissue growth rate, can be accurately controlled, this enabling the 'artificial bone' to be gradually replaced by natural bone tissue.

Laboratory studies on a rabbit-bone/artificial-bone join have so far produced very encouraging results. This photomicrograph, taken two months after implant, shows that a great deal of resorbtion has taken place. There is no evidence of any onset of rejection: no fibrous encapsulation tissue is found, and newly formed bone has been deposited directly to the ceramic implant. Osteoclast-like cells can be seen, as well as an osteon-like newly created pore complete with blood vessel.

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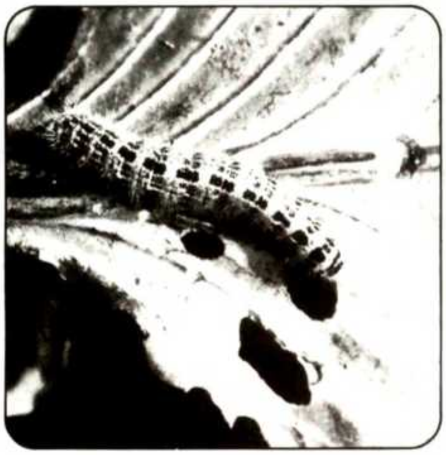

Crop protection. DIMILIN, a very selective insecticide made by Philips-Duphar, has recently been registered by the US Environmental Protection Agency for the control of the cotton boll weevil (Anthonomus grandis), which causes extensive damage to cotton crops. On cotton crops, for example, DIMILIN affects the boll weevil only and does not kill other insects. Unlike many other insecticides, adult insects are not killed. Since DIMILIN does not penetrate plant tissue, sucking insects will also not be affected. These characteristics form the basis of the unique selectivity of this insecticide. A further advantage of DIMILIN is, that only very small quantities are required to protect a crop: in cotton culture about 50 to 100 grams per hectare, applied six times per season.

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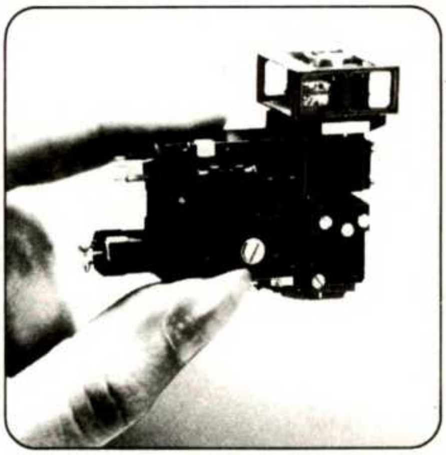

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Nuclear secrets: no clear frontiers

WHAT is a military secret? And how does it lose its secret status? Until a few years ago, the answers to these questions were thought to be relatively straightforward — a secret was information with the word 'secret' stamped on it; it ceased being a secret when the word 'secret' was removed. But the public, or at least a portion of it, is no longer quite so trusting of military judgement and recently even the holy of holies — details on the construction of nuclear weapons — has been violated in various publications, most notably the article on hydrogen bombs by Howard Morland that has just appeared in *The Progressive*, and the long open leter to US Senator Charles Percy from Charles Hansen, a Californian computer programmer whose pastime is the study of nuclear weapons.

Publication of these documents has been accompanied by some ringing statements about press freedom. For instance, in printing the Hansen letter, the *Chicago Tribune's* Publisher Stanton Cook declared: "We are convinced it contains no secret information that would in any way jeopardise the national security. We are confident that the information was gathered from public records available to any researcher . . . The government's attempt to muzzle a private citizen and the press (is) repugnant to the First Amendment's guarantees of free speech and press . . ."

Now Mr Cook is right (or almost) on two counts. It is indeed possible to accumulate information that is in the public domain and come up with some sound conclusions concerning the design of nuclear weapons. And the revelations of the last month are certainly not going to put the United States at risk — at least in the near future. But to go on to claim freedom of speech and of the press as justification for broadcasting of nuclear weapons data could, just conceivably, be a serious misuse of these fine but not absolute principles.

Consider first the question of the accumulation of public information. There is a serious danger in the view that something is either totally in the public domain or totally secret. There is almost no secret which cannot be deduced if a researcher is prepared to devote enough time, effort and money to the sifting and collating of evidence. The only thing the holder of secrets can do is to make it immensely difficult to extract those secrets. There is thus no great merit in the case that a secret is no longer a secret when it can be deduced from the public domain. It may still be worth keeping the price of learning the secret very high.

But, it may be argued, there is no point in keeping the secret any longer, either because the US government shouldn't be making nuclear weapons (as Mr Morland might argue) or because the US government should go on making weapons, but for other people to know how won't harm the United States (as Mr Cook might argue).

This is to act in ignorance of the Non-Proliferation Treaty. The treaty to which the United States is party, declares that nuclear-weapons states will not in any way assist, encourage or induce any non-nuclear-weapon state to acquire nuclear weapons. It is, of course, debatable whether the two documents in question do 'in any way assist', but simply because there are sincerely held views that they do, there seem powerful reasons why the narrower question of American security should yield to the broader one of global non-proliferation.

None of this should be seen as a blanket endorsement of military secrecy, much of which is preposterous and merely classifies material of low quality to protect it from critical scrutiny by outsiders. Nor should it be seen as in any way a retreat from the view that matters of nuclear policy (as opposed to hardware) are inadequately debated in public, because of the paucity of policy information available, particulary in the United Kingdom. It is simply a statement that in a difficult field where there are no clear frontiers, the case for the recent publications is not as strong as might seem at first sight. □

Overpaid humanitarians

MANY scientists in Britain speak with justifiable resentment about the tax-free salaries that employees of international organisations command. People subjected to no more privation than to live in another country (and sometimes not even to have to learn the language) are frequently paid sums which seem grotesque by continental European and US standards, let alone by those of poverty-stricken Britain.

The latest post to come to our attention is that of Chief of Public Information for an 'International Humanitarian Organisation in Geneva' (which prefers to remain anonymous). The holder of this position will, to be sure, have to be fluent in French and English, and will have to act as spokesman for the organisation running a department of fifteen. The annual salary is round $55,000 to $60,000 — net.

There are highly dedicated information officers in many national humanitarian organisations around the world who count themselves lucky to receive a quarter of this sum — gross. International organisations, particularly those connected with humanitarian aims, have gravely lost their sense of direction when they offer enormous salaries. □

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1979 Nobel Prizes for Medicine, Physics, and Chemistry

35th prize for inventor of EMI X-ray scanner

"I'm not the sort of person to build model planes" said Godfrey Hounsfield, co-winner of this year's Nobel prize for medicine, last week. "Ive always searched for original ideas; I am absolutely opposed to doing something someone else has done."

Dr Hounsfield invented the EMI 'brain scanner' for computerised tomography — using X-rays to make two-dimensional images of slices of a patient's brain. "It came to me on a ramble" he said. The device, and its successor, the whole body scanner, brought EMI medical division a ten-fold increase in profits from 1973 to 1977, when the US market collapsed from 40 scanners a month (at £250,000 each) to just eight (see following story).

To date, Dr Hounsfield has received 35 academic awards for his work on the scanner, beginning with the MacRobert Award (the UK's major prize for engineering) in 1972, a Fellowship of the Royal Society, a CBE and many foreign degrees, and ending with the Nobel. He hopes to use his £44,000 prize to buy scientific equipment for his home "so I have something to do when I retire".

Sixty years old and a bachelor, Dr Hounsfield hasn't much time for the arts. "Science has very much a 3rd place in this country; I'd like to do something about that. The sciences are far more exciting than the arts, than fantasy".

Farming educated Hounsfield. "I was brought up in the country; I played around with farm machinery, and to prevent myself being bored I started to reason why things work".

EMI's central research laboratories have provided Hounsfield with "a very relaxed place to do original thinking; and there's just enough pressure to produce a product. It enables one to come to a logical conclusion rather quicker than one would in academic life".

Dr Hounsfield does not know his co-winner, Allan Cormack, and says he first heard of him when he was recommended as a referee for a paper in 1972-3. Cormack had taken a mathematical approach to computerised tomography, whereas, says Hounsfield "I find I've got other tools of thinking than maths — the most important thing is a very broad understanding of the problem, not to be fogged by detail".

"In the last few years I've spent half my time on developments of the scanner, half on other things" says Hounsfield. A new version, the '7000', will cut scan time from 18 to 3s, so reducing the effects of organ movement in the body. "I've always been interested in the heart; we are working on pictures synchronised to the heart to 'arrest' the heartbeat."

Dr Hounsfield has also been working on newer, non-invasive techniques, such as nuclear magnetic resonance. This "looks promising, but isn't diagnostically useful yet. But we've a long way to go before we reach a brick wall." Further developments of the scanner involve a system directly linked to a machine for delivering therapeutic X-rays, so the surgeon can see precisely where he is working. "We're well ahead on that".

Godfrey Hounsfield: started with farm machinery

"The scanner can save money if it's used properly" says Hounsfield. "It's said the head machines can pay for themselves in 2 years" by avoiding the need for exploratory surgery or dangerous techniques like pumping air into the ventricles or heavy metals into the veins of the brain, which are the more traditonal techniques for revealing defects.

Nevertheless the profits of EMI medical electronics have fallen from £14.7m in 1977 to a loss of £12.8m this year, in part because of the spectacular collapse of the US market for the scanner. And meanwhile EMI has been fighting patent rights battles with three US manufacturers of scanners: Ohio Nuclear, which is now to work under an EMI licence, and Pfizer Inc., and GEC.

Robert Walgate

● A six-month tenure at the Groote Schuur hospital in Capetown gave Allan Cormack, a native South African, first-hand knowledge of the limitations of the X-ray techniques being used. "I saw these girls

Dithering on the deadline

PROFESSOR Cormack and Dr Hounsfield became Nobel laureates after a controversy that prolonged the announcement of the prize by more than an hour. Until the last minute, it was unclear whether they or another group would share the award.

The rival nomination was reportedly for a group of three American and French scientists who have made crucial discoveries in immunogenetics. As a result of these discoveries, organ transplant have been made easier.

Exactly who these scientists are is not being revealed. But the Nobel committee, a group of 15 professors from the Karolinska Institute which goes through all the nominations and prepares a short-list, was so sure that the group would be approved that it had prepared its publicity material only on the immunogeneticists, in the usual languages: Swedish, German, French and English.

When the committee presented its proposals to the Nobel Assembly for final decision, however, the Assembly — composed of the 64 full professors at the Karolinska Institute — argued in favour of Cormack and Hounsfield instead. After a prolonged discussion, the committee's secretary, Professor Jan Lindsten, left the deliberations to face the waiting journalists. He made the official announcement in Swedish only: the translations came later. "There was no time to check with the translators" he told *Nature* — and the foreign correspondents had to manage as best they could.

In voting for Cormack and Hounsfield, the Assembly decided to give the prize to applied instead of basic research. The relative merits of these was reportedly one of the subjects of the Assembly's discussion. The information secretary of the Royal Academy of Sciences, Lennart Daleus, wondered afterwards how such a controversy could have arisen at the last minute. By the time the Academy's classes of physicists, chemists and economists present the full Academy with their proposals for the respective prizes, he mused, all the substantive disagreements have usually been ironed out.

Nominations for the medicine prize are invited from many sources. According to Professor Lindsten, invitations to nominate are sent to previous laureates, professors of Scandinavian faculties of medicine, and about 40 faculties of medicine in different parts of the world.

After the drama of the announcement, none of the Assembly's members was willing to talk about the controversy. The remark made by the Assembly's chairman, Professor Georg Klein, was typical of the others' tantalising restraint. "I have a lot of comments", he told *Nature*, "but I can't make them."

Wendy Barnaby

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preparing all these X-ray treatments, and as a physicist, it struck me as a very rough process," Dr Cormack said. "How can you know how to treat something in the middle of the head if you do not have proper information of the effect on the radiation of the material through which it has to pass?"

The question appealed to him as a scientist. But the task of working out the answer was "mostly a mathematical one", extrapolating what physicists already knew about the attenuation of radiation passing through a homogenous material to similar calculations for inhomogenous materials.

"After publication in 1963 there was virtually no response at all, except for a few physicians, until someone came along in about 1970 to say that similar ideas had been developed by engineers at EMI," says Mr Cormack. He says that he has never met Godfrey Hounsfield, with whom he now shares the Nobel Prize, but adds that "I have heard that the is an inventive person."

Cormack's lack of involvement with the commercial development of computerised comography (CT) scanners means that he has been able to watch from the sidelines the intense debate which has taken place in the US — and which promises to be revived by the Nobel Prize award — over the use of the technology. In particular, this debate has focussed on the extent to which the federal government should support the growth of a technology whose extremely high capital costs have contributed to the rapid increase in hospital care costs in recent years.

Mr Cormack is critical of the US government's decision to place stringent restrictions on the use of CT. This move followed a report from the National Academy of Science's Institute of Medicine pointing out that in many cases, conventional X-ray techniques were much cheaper and might be just as effective (*Nature,* 12 May 1977).

"The arguments against the CT-scanner leave out a lot of things. In doing a cost-benefit analysis, for example, how much to you put in for the pain and suffering of patients for whom exploratory surgery might be avoided by using the scanner?" Mr Cormack says.

"I think the scanner has become a symbol, it is used as a whipping boy as a way of pointing out the expense of medical care; intensive care units probably cost a lot more money, but you do not usually hear about them."

At the same time, however, he admits that the cost of the basic equipment might be brought down if manufacturers were not under great pressure from physicians to improve its performance, for example through faster scans or higher resolution. "A substantial part of the costs must still be in development. If they could standardise the model, the costs would probably go down a lot," Mr Cormack says.

David Dickson

Neutral currents bring rewards

THIS is the prize that many physicists have been waiting for — not least Abdus Salam, of Imperial College, London, and the International Centre for Theoretical Physics, Trieste, and Steven Weinberg of Harvard, whose unified model of the action of the weak and electromagnetic forces has been finding corroboration in an increasing number of experiments.

But one of the severest tests of the theory — the discovery of heavy counterparts to the photon, the 'intermediate vector bosons', is yet to come, and a few will no doubt consider the prize to be premature. Experiments planned at CERN, the European centre for subnuclear physics, for 1982 are expected to provide the first direct evidence of the particles.

However the citation refers to the prediction "*inter alia*" of neutral currents, and here the theory is on firm ground. Sheldon Glashow, of MIT, played a key role in their prediction independently of the machinery of the Salam-Weinberg model, and hence his well deserved share in the prize.

The term 'neutral current' is a somewhat confusing epithet for weak interactions not involving the exchange of electric charge. For example, the familiar weak decay of atomic nuclei involves neutrons changing into protons (or *vice versa*), the excess charge being taken up by the creation of an electron and an antineutrino (or their antiparticles). This is supposed to take place by the emission and reabsorption (exchange) of a *charged* intermediate vector boson between the nucleon and electron systems. Neutral current interactions, on the other hand, involve the exchange of a neutral IVB, and so change protons into protons, electrons into electrons, and so on.

The weak force being extremely weak, the effects of these 'neutral' exchanges are completely masked by the analagous exchange of photons which creates the electromagnetic force between, say, protons and electrons. (It is this analogy, indeed, which allows the two forces to be unified in an all embracing theory.)

The detection of neutral currents has thus proved extremely difficult, and its history has not been without its conflicts of evidence. The first observation was made with neutrinos (where electromagnetic effects are absent) at CERN; others followed, and gradually the neutrino evidence has built up into an impressive body of evidence not only for the existence of the neutral current, but for the exact form of it predicted by Glashow and incorporated by Salam and Weinberg in their (independently generated) 1967 and 1968 unified theory. More recently, a brilliant experiment with polarised electrons at the Stanford Linear Accelerator Center in California measured the interference between the electromagnetic and neutral weak interactions between electrons and protons, and also confirmed predictions.

Robert Walgate

Boron and phosphorus

Herbert C Brown of Purdue University, Indiana, and Georg Wittig of Heidelberg University share the Nobel Prize for chemistry. The citation describes their work on the application of boron and phosphorus compounds respectively to synthetic organic chemistry.

Professor Wittig, who is now over 80 but still academically active, is best known for the development of Wittig reagents, a class of phosphorus-based compounds used to form carbon-carbon double bonds at precisely predetermined places in organic molecules. They have applications in the synthesis of many compounds, including prostaglandins.

Professor Brown, on the other hand, has developed a series of very versatile organoboron compounds which can selectively modify functional groups in organic molecules, and is also renowned for his theoretical contributions to carbo-cation chemistry. His birthplace was in the UK, but he has pursued his career in the US. ☐

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Fellowships proposed to help US jobs problem

THE US National Science Foundation should be prepared to spend up to $30 million a year over the next twenty years to reduce the potential impact of demographic changes on the quality of university research, according to a report published last week by the National Research Council, the research arm of the National Academy of Sciences.

Addressing a problem of increasing concern both to research scientists and to science policy makers, the report suggests that the NSF set up a new fellowship scheme to provide support for up to 250 university science faculty members for a period of five years.

In return, the scientist's university would be required to use the money which would have been used to support the scientist to recruit a new faculty member. The report says that the total cost of the programme would be about $381 million (at 1979 prices) over a 20 year period, a sum which would represent about 3% of the NSF's annual budget. After 20 years it predicts that a steady state will have been reached with faculty recruitment balancing retirement and that federal intervention will no longer be necessary.

There are two particular aspects of current demographic changes which threaten — and in some cases are already exerting — a considerable constraint on the ability of US universities to produce high quality research, says the report: declining student enrolment and a low level of faculty retirement in a number of scientific disciplines.

The report points out that whereas in the mid-1960s higher education institutions were expanding at an average rate of 13.2%, the current rate of expansion is only about 3%, and with a rapidly falling number of 18 year olds in the population, "the prospects are very real that such enrolments will actually decline". This has inevitably led to a decline in the rate of faculty growth, it says.

An even greater impact on research, however, is likely to come from the current low retirement rate. This is partly due to the fact that universities expanded rapidly in the 1960s, after a relatively slow build up during the previous decades, and that faculty members recruited at this time are still far from retirement age — and to the impact of new legislation, which will make it illegal after 1 July 1982 for universities and colleges to establish a mandatory retirement age of less than 70.

"It is not known what proportion of faculty who under previous law would have been required to retire at age 65 will instead choose to stay on longer," the report says. It takes some pains to point out that the situation differs between disciplines, the shortage of retirements for example being most severe in fields such as physics and mathematics. "We are concerned that a number of reports in the past have over generalised the problem and are pleased to note that there are some fields, such as the agricultural and earth sciences, where this is not likely to be a problem," Dr Robert M Bock, chairman of the committee which produced the report and dean of the Graduate School at the University of Wisconsin, Madison, told *Nature* last week.

However, the report says that overall, faculty retirement over the next five years will be about 5.5% of the total. A steady state model (which is expected to be reached in the mid 1990s) would imply a retirement rate of 9.2% over this period. "Thus approximately 2900 fewer positions will become available through retirements at PhD granting institutions over the next five years than would in the steady-state with the same total faculty size."

The major effect of these two trends, says the report, will be a reduction in various fields of the number of young faculty research workers. And this is potentially serious for the effectiveness of academic research, not because such research workers have a higher productivity, but because they play a vital role in introducing new ideas and perspectives into the research community.

Furthermore, the report says that "a sharp reduction in the rate of hiring of new PhD faculty might pose a serious threat to the continuation of the progress that has recently been made in expanding the participation of women and minorities in science and engineering faculties." Thus unless ways are found to ensure a continued flow of young scholars into academic positions during the transition to a steady state, "academic research in the United States is likely to suffer serious erosion in the coming decade".

Having argued the case for federal intervention, the report weighs up the arguments for the various forms that this intervention might take. Direct federal support for university faculty positions would, it says, interfere with the normal university process for selecting assistant professors. Similarly effective early retirement and career change programmes can best be implemented and designed at the level of the individual institution.

It also argues against expanded federal postdoctoral fellowship programmes. It states that "post-docs play an enormously valuable role as producers of research, but they cannot be looked to as the principal sources of innovation and renewed vitality in academic departments". Similar reasons are given as arguments against expanding the pool of non-faculty researchers as an adequate response to what is referred to as the "vitality problem".

Setting out the various criteria required for such a response, the report says that these are best met by a programme which provides research awards in support of salary for existing faculty members for a five year period, and requires the university to use the funds thereby released to hire additional faculty.

It therefore proposes that the NSF develop a research excellence award programme, nominees for which must be full-time faculty members on regular appointments. Any nomination made for such an award would have to be accompanied by a staff development plan prepared by the department and endorsed by the university.

"This would be a considerable departure from current NSF practice under which a senior faculty member can win salary support for summer research, and sometimes for a complete academic year, but almost never for a five-year period. Nor is there currently any commitment by the university to use the funds freed in this way to employ new faculty members," says Dr Bock.

No official response has yet been given to the report by the NSF, but it is expected to announce a programme soon.

David Dickson

Research Excellence through the year 2000 Office of Publications, National Academy of Sciences, 2101 Constitution Avenue NW, Washington DC 20418. $9

Age distribution and anticipated retirements of full-time faculty at PhD granting institutions, by field of science and engineering, 1977

	Percent of Full-Time Faculty With Age			Anticipated Retirements as Proportion of Steady-State Retirements		
	> 60	56-60	51-55	1978-82	1983-87	1988-92
All fields	5.5	8.1	11.5	.60	.66	.88
Mathematics	4.5	4.2	7.6	.49	.34	.58
Physics	3.3	7.0	13.5	.36	.57	1.03
Chemistry	8.0	8.0	8.5	.87	.65	.65
Earth sciences	7.4	7.0	14.0	.80	.57	1.07
Engineering	4.0	7.8	13.2	.43	.63	1.02
Agriculture	7.5	14.2	14.0	.82	1.15	1.08
Biosciences	5.9	8.0	12.1	.64	.65	.93
Psychology	4.7	6.6	9.9	.51	.54	.76
Social sciences	6.2	8.8	11.1	.67	.72	.85
Steady state	9.2	12.3	13.0			

Source: NRC, Survey of Doctorate Recipients.

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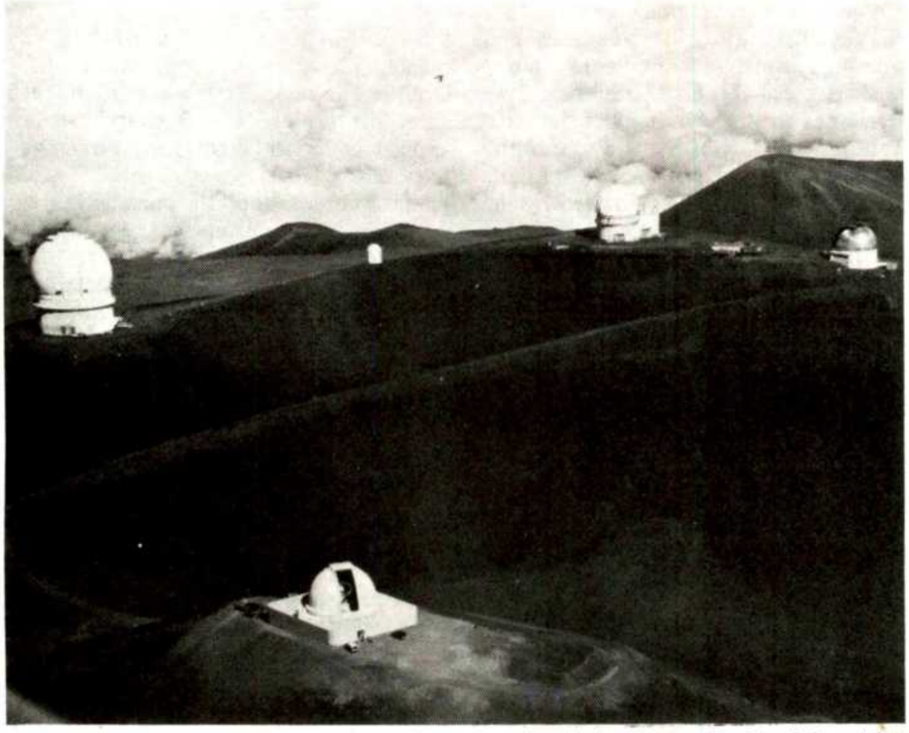

On top of Mauna Kea: In the foreground is the NASA infrared telescope; on the ridge (left to right) the Canada-France-Hawaii telescope, the 2.2m Hawaii telescope, and the latest arrival, UKIRT

Britain joins Hawaii summit

THE United Kingdom Infrared Telescope (UKIRT) at the summit of Mauna Kea on the island of Hawaii was opened by HRH the Duke of Gloucester on 10 October. Funded by the Science Research Council and operated by the Royal Observatory, Edinburgh, this 3.8-m diameter instrument is the largest telescope in the world designed specifically for infrared observations.

The UKIRT, however, is not the only telescope to have been built recently on Mauna Kea. The NASA 3m Infared Telescope Facility, and the 3.6m Canada-France-Hawaii Telescope have also opened within the past three months, joining three smaller University of Hawaii telescopes already on the summit. In terms of collecting area, Mauna Kea now houses the world's largest observatory for optical and infrared astronomy.

Infrared astronomers will be looking with particular interest at the relative performance of the UKIRT and NASA telescopes, since their design philosophies are significantly different. The British instrument goes for maximum collecting area while in the NASA telescope, which cost about twice as much as the UKIRT, image quality and guiding stability are given the highest priority. The Canada-France-Hawaii telescope and the University of Hawaii 2.2-m telescope are also instrumented for infrared observations.

The UKIRT telescope cost £2.7m and was built by the Sheffield company of Dunford Hadfields, Ltd, using a Cer-Vit primary mirror which was ground and polished by Grubb Parsons, Ltd, of Newcastle. An important feature of the design of the telescope is the thinness of its mirror, which weighs only 40% of that of a conventional telescope of the same aperture. As a result, the whole instrument was built at very substantial saving of weight and cost as compared with, for example, the similar sized Anglo-Australian Telescope. The lightweight structure has the disadvantage of lesser rigidity, but careful design and the use of computer controlled pointing has resulted in an instrument whose performance approaches that of the best optical telescopes.

The instrumentation for the telescope, built in Edinburgh, includes photometers and low resolution spectrometers covering the wavelength range 1 — 40μm. The detectors in use are cryogenically cooled solid state devices such as indium antimonide photovoltaic detectors or gallium-doped germanium bolometers. Stray thermal infrared emission from the sky and from the telescope itself is a major problem in infrared astronomy; the optical system has therefore been designed to minimise the size and effective emissivity of the mechanical and optical components that lie in the telescope beam. In order to separate the signal due to a celestial infrared source from the background emission the secondary mirror itself oscillates at up to 40 Hz, providing the detector with near-simultaneous images of two adjacent areas of sky.

Gareth Wynn-Williams

Gareth Wynn-Williams is Associate Professor of Astronomy at the University of Hawaii

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Coast guards probe mystery of missing research ship

WITHIN the next few weeks, the US coast guard hope to have developed a plausible explanation for the mysterious disappearance last December of a research vessel charted by the University of Hawaii with seven scientists on board.

No trace of the vessel, the Holoholo, has been found since it set sail from Honolulu harbour on the afternoon of 9 December. The trip was one of a series planned to gather data on the oceanographic conditions off the Hawaii coast in connection with the siting of a proposed ocean thermal energy conversion (OTEC) project supported by the Department of Energy.

Evidence has been found to indicate that the vessel may have put in to shore to make bench-mark readings for some of the scientific equipment. However, the alarm was raised when it failed to make a planned rendezvous with two scientists two days after its departure from Honolulu, following a period of stormy weather.

On board the Holoholo at the time of its disappearance were three scientists from the University of Hawaii — including the principal investigator for the project, assistant professor of engineering Dr Gary C Niemeyer — two scientists from the University of California's Lawrence Livermore Laboratory, which has responsibility for the OTEC research programme, and two scientists from the National Oceanographic and Atmospheric Agency.

Various theories about the disappearance have been voiced during extensive hearings that have already been held in Honolulu by a board of enquiry established jointly by the coast guard and the National Transportation Safety Board. These range form the hypothesis of an explosion on board to the suggestion by some, quoting the Glomar Explorer's links with the CIA, that the vessel might have been captured by a foreign power.

According to a local newspaper, the Honolulu advertiser, the board that the boat sank after being swamped by heavy seas — and that the swamping may have been partly due to structural alterations made to the vessel to accommodate various species of equipment required for the research.

The safety board has already issued a set of recommendations to the University following the Holoholo's disappearance in which it says that some of the alterations, which included the installation of two large winches, a large spool of cable, and an A-frame cargo boom on the after part of the vessel, "may have adversely affected the

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seaworthiness of the vessel".

The board points out, for example, that although the chief scientist principal scientist was "a highly regarded investigator with considerable knowledge and experience in ocean research", there was no evidence that he or the other scientists had expertise in vessel seaworthiness, stability, or navigation.

The board also raises questions with regard to the adequacy of procedures adopted by the university for observing guideline safety standards for research vessels laid down in may 1976 by the University National Oceanographic Laboratory System (UNOLS), based at Woods Hole in Massachusetts, an Association of Oceanographic Institutions.

It points out that although these include "an excellent array of standards and procedures which would be applicable to most research vessel operations", and despite the fact the University of Hawaii is member of UNOLS, the guideline standards had not been applied to the Holoholo, which was not owned by the university but had been commissioned through a subcontract with the university's research corporation.

As a result, says the board, the operation of the Holoholo apparently relied on the charter contractor for vessel seaworthiness, on the contract with the boat's master — of whom there is no evidence or expertise in vessel stability — for safe operation, and on the judgement of the chief scientist as to the suitability of the vessel to serve as a platform for the research projects to be conducted.

"There is no evidence that any of the guidelines standards were considered or applied by three individuals involved or by any other officials of the university or research corporation" the board says.

Officials at the university, which may be sued for substantial damages by the relatives of those on board the vessel when it disappeared, have declined so far to make any public statement about the incident, apart from saying that the specific recommendations of the safety board to prevent similar occurences in the future" are now under study by university officials. **David Dickson**

Drought could turn Dead Sea into a desert

Dead Sea: getting deader

CROSSING the Dead Sea on foot is now theoretically possible — if it weren't for the presence of border patrols — according to Shlomo Drori, a leading Israeli expert on the use of Dead Sea resources. Following the worst drought in the history of the State of Israel, the sea has split in two at its narrowest point.

The Dead Sea's sole feeder is the lower Jordan, which is becoming increasingly depleted by water developments, both Israeli and Jordanian. Last November, even before the onset of the drought, Yigal Allon, the former Israeli foreign minister spoke in the Knesset of the "urgent" problem of the Dead Sea. Current Jordanian water projects involve the damming of all eastern tributaries of the Jordan; when these are completed, said Mr Allon, the lower Jordan would totally disappear save as a drainage channel, the Dead Sea level would fall signifacantly, and the southern part of it would become a salt desert. This, as the Energy Minister Yitzak Moda'i admitted in his reply to Mr Allon, could cause "tremendous" and possibly irreversible ecological damage.

It is, however, Israel's water schemes which are the main consumers of sources which would, without human intervention, end up in the Dead Sea. For the lower Jordan flows from the sea of Galilee, which, via the National Aquifer, serves as the water source of the whole country. According to Professor Yoram Avnimelech, a soil chemist seconded from the Technion to the Sea of Galilee Conservancy Board, considerable problems are posed to conservationists by the unique status of the Sea of Galilee, including its religious and historical associations. The normal way of treating a lake scheduled as a drinking water source, he told *Nature,* is to fence it off, minimise development of the watershed, and try to keep the population out. This, however, is not possible in Israel, which has a limited supply of water — indeed, in the summer, every drop of water from the springs of the Upper Galilee is used three times by irrigation and fish-farming schemes before it even reaches the Sea of Galilee.

On occasion, the needs of the Conservancy seem to run counter to Israeli national sentiment. In 1968, workers at the Limnological Laboratory at Tevhag began to observe excessive levels of nitrates in the water, due not only to the use of fertilisers in the Upper Galilee but also to the uncovering of the organic soils of the Huleh valley. Since the reclamation of the Huleh marshes has attained a saga-like status in Israeli history, the obvious alternative of reflooding the Huleh was simply not acceptable, but it took several years of intensive research before a suitable strategy was devised to obviate the danger of eutrophication posed by the excess nitrates. (This is based, incidentally, on short-term sprinkling of the Huleh soil at high summer temperatures to encourage the action of denitrifying bacteria).

Professor Avnimelech, who is very much the scientific trouble-shooter for the Sea of Galilee Conservancy, also indicated another area where Jewish sentiments and the needs of conservation may yet clash. "Planting a tree in Israel" is a tradition with considerable emotional overtones to Israeli and Diaspora Jews alike — yet recent indications, Professor Avnimelech said, suggest that over-zealous tree-planting in the Galilee watershed may be causing environmental deterioration.

Yet, however well the Water Conservancy Board husbands the Galilee resources in times of drought, the Sea of Galilee itself may be at risk. This summer, the water level fell dangerously low, and when the rain finally fell just before Jewish New Year, one official revealed that no water had been taken from the Sea of Galilee for almost four months — the country had been subsisting solely from supplies stored in auxiliary reservoirs along the aquifer.

As far as the depleted — and now divided — Dead Sea is concerned, there does exist one fairly obvious means of replacing the waters lost to human needs. This is the construction of a canal to bring in water from the Mediterranean. Israel, of course, does not have the funds to contemplate such a scheme merely as an ecological rescue operation. However, the Dead Sea lies some 400 m below the level of the Mediterranean, so that, the canal could descend via series of hydroelectrical cascades which could according to current calculations, produce up to 10% of Israel's

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present generating capacity. Although, according to Energy Minister Moda'i, electricity can still be generated more cheaply using fossil-fuelled power stations, granted the need for such a canal, power-production might make it economic.

One early exponent of such a canal was American pioneer of land conservation, William Clay Lowdermilk. In 1939, Lowdermilk formulated an "eleventh commandment" for the future state of Israel. This reads, in part: "Thou shalt safeguard thy fields from soil erosion, thy living waters from drying up . . . .". This summer's drought has made it imperative that this "commandment" should be extended to include not only the "living" waters of the Jordan and Sea of Galilee but also those of the Dead Sea.

Vera Rich

UK plans nuclear expansion

BRITAIN'S Conservative government is expected to announce details of a £10bn nuclear expansion programme within the next few weeks. The plan will involve building up to 20 nuclear reactors to supply half Britain's electricity needs by the year 2000. The Conservative cabinet is unanimous in its support for nuclear power, the only disagreements being over reactor type.

At present it is believed the American pressurised water reactor of the type involved in the Harrisburg accident is the leading candidate because it can be brought on line in five years, half the time needed by the advanced gas cooled reactor. Two public inquiries will be held before the plan can be enacted. One is a planning inquiry about the siting of the PWR, the other a full public inquiry about the necessity for a fast breeder reactor for Britain's nuclear future. Although strong opposition plan to counter it. "They will wait and see how effective the opposition is before taking counter measures" a Department of Energy spokesperson told *Nature*.

USSR questions nuclear safety: For the first time, the Communist Party has published an article on negative environmental effects of nuclear power. The article, in *Kommunist*, the party's theoretical journal, was co-authored by Nikolai Dolezhal, a prominent nuclear scientist, and Yurii Kroyakim, a relatively unknown economist. It questions the amount of valuable agricultural land that would be used, the effect on water supplies and the difficulties of storing nuclear wastes.

The article stresses that the sheer scale of the programme involving an expansion from the present level of 10 million kW to over 110 million kW by 1990 plus the construction of nuclear power stations in western Soviet cities as sources if district power heating would encroach considerably on agricultural land. Previous Soviet comment has characterised the anti-nuclear movement as being inspired by oil companies anxious to preserve their market position.

French demo blocks fuel: In a militant demonstration against nuclear fuel reprocessing, French trade unionists and environmentalists held up the unloading of 15 tons of spent nuclear fuel from Japan. Nearly 2,000 demonstrators met the ship chartered by British Nuclear Fuels Ltd when it docked at Cherbourg on 9 October with its cargo for reprocessing at nearby La Hague.

Demonstrators blocked a railway siding, severed an electric cable and put a crane out of action in a successful attempt to block the unloading. The Socialist Party and the socialist trade union federation the CFDT are demanding a national debate to prevent France from becoming "the nuclear dustbin of the world".

Waste disposal plan could take a decade: An independent waste disposal plan produced by the state of California has caught the nuclear industry by suprise. The plan calls for simulated waste to be placed in experimental vaults 1000 metre under ground in variety of geological formations. Measurements would be taken on migration rates as a function of rock type, temperature, depth, and pressure and the findings compared to theoretical predictions.

The entire process would take ten years and is recognised to be the only valid way to assess present underground storage plans. The federal government accused the state government of ignoring national energy policy and of failing to keep Washigton informed. The move is widely regarded as a show of strength by the anti-nuclear movement and is expected to give a boost to California governor Jerry Brown's campaign for the presidency.

Protestors march in Bonn: In the largest demonstration since the early 1930s, protestors demanded an immediate end to the development of nuclear power is West Germany. Individual columns stretched more than 2½ miles as 100,000 activists including representatives from the US Austrian, Danish, Dutch, French and Swedish anti-nuclear movements converged on Hofgarten park in central Bonn last Sunday. Demonstrators gave a standing ovation to a speech from Kathy McCaughin of the Harrisburg Citizens Action Group who called for a stop to "the nuclear madness now". Trade union speakers were applauded when they said that more jobs would be created by alternative technology instead of nuclear power.

Joe Schwartz

Syrians blast UNCSTD over power politics

SYRIA'S delegation to UNCSTD returned home unsatisfied and a little angry. "We know that the problems of development cannot be solved with one conference, but at least we could have made a start", says Dr A W Chahid, Director of the Centre for Scientific Studies and Research in Damascus and a leading member of the Syrian delegation.

The future for the developing countries after UNCSTD, says Dr Chahid, is "just as hazy as it was before. We came back from Vienna even more convinced of the inherent biases and injustices of the existing scientific and technological order in the world".

Most of the developing countries were not prepared for the tight, hard-nosed negotiations and power play that was conducted by the developed countries at UNCSTD. Dr Chahid is particularly critical of the petty politics and the infighting of the European Community countries. "The attitude of the Nine to the proposed Development Fund was quite pathetic", he says. (A fund of $2 billion to 1985 proposed by the Group of 77 was cut to $250 million to 1981.) The Nine were also responsible for frustrating the good intentions of "the Nordic Group which was quite forthcoming and ready to make a reasonable contribution to the fund".

Dr Chahid thinks it was unfortunate that the Group of 77 was dominated by countries that are at the top of the development continuum. India, Brazil, Yugoslavia and other members of the 'Group of 27' of the Group of 77 who took part in the negotiations have a reasonably developed scientific and technological infra-structure. "It is these countries which stand to gain most out of the few achievements of UNCSTD".

But what about the really poor countries that suffer from the "small country problems", that do not have even the tiniest form of science and technology infrastructure? "What do they get out of UNCSTD?" asks Dr Chahid. "We must ensure that they get the lion's share of the Development Fund, little though it is".

The power and coordinating ability of the Group of 77 will be tested to the full in the setting up of the UN intergovernmental committee that will administer the Development Fund. Dr Chahid argues that if all members of the Group of 77 took an active interest in the intergovernmental committee and if there was "a very strong Group of 77 representation on the Committee", it may be possible to steer development in the Third World's favour.

However, Dr Chahid points out that many developing countries, including Syria, do not have a representative in New York.

Ziauddin Sardar

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news in brief

Western nations increase proportion of R & D funds spent on military research: The proportion of research and development funds spent by the United States and Western Europe for military purposes increased from 38.5% to 39.2% between 1975 and 1977, according to a report published last week by the US Arms Control Association and the Institute for World Order. The amount of money spent on military research increased from $12.8 billion to $15.4 billion, says the report, which points out that the global expenditure on military purposes exceeded the rate of inflation for the seventh year, reaching a total of $425 billion.

The report says that the R & D component of military spending is significant not only for what it has produced, but also for the continuing momentum that it gives to the arms race generally. "The steady improvement in technology has made the pace of national military spending essentially independent of the existing threat to the nation."

"In the superpower competition, each side considers it essential to develop and produce whatever is technologically possible, on the grounds that the adversary may do so. R & D is the instrument through which 'antagonistic cooperation' between the two leading adversary nations ensures a never-ending escalation of arming."

World Military and Social Expenditures 1979, by Ruth Leger Sivard. Single copies $3,50. Available from Work Priorities, Box 1003, Leesburg Va. 22075, US.

Czechoslovakia considers ecology of uranium mining: Czechoslovakia's Minister of Energy Vlastimil Ehrenberger announced last week that the country's uranium industry had met all its increased production targets. Attention was also being given to the ecological aspects of uranium mining. In a 40-minute speech on the state of the energy industry, he told the Chamber of the People that new uranium ore deposits now ensured a "reliable base" for the country's nuclear energy industry. A large ore-processing plant, built with Soviet assistance was operating in North Bohemia. Exports of uranium to the Soviet Union were well up to target, and a scheme is being worked out for the future of the uranium industry after 1980, when the agreement signed in 1945 for supplies to the Soviet Union, runs out.

Rattled: Pennsylvania rattlesnake steaks are no longer on the menu at Dominique's restaurant in Washington DC. The rattlesnakes have been spared as the result of a letter to the restaurant's owner by C.K. Dodd, an endangered species herpetologist at the US Department of the Interior. But Mr Dodd's intercession on behalf of the Pennsylvania rattlesnakes may have cost him his job. US Secretary of the Interior, Cecil D. Andrus, a patron of the restaurant, apologised to the owner Dominique D'Ermo, for his employee's action assuring D'Ermo that the snake "is not listed as either threatened or endangered under the Endangered Species Act'; and Mr Dodd received a four page latter of dismissal.

The most serious charge against Mr Dodd seems to be that the wrote his letter on Department of the Interior Stationery. He admitted that he might have been at fault, but claimed "Even if it is against the rules most people would get a simple slap on the wrist or a reprimand". He plans to appeal. *Anne Norman*

Vietnamese claimed to use poison gas: US representative Jim Leach released a report last week which claims that a number of Hmong refugees had witnessed or had been victims of seven poison gas attacks in the past year. The Hmong are a hill tribe in a remote area of Laos. They were an ally of the French and then the Americans during the wars in Indochina. According to the report, refugees from the tribe described poison gas attacks in which gas was dropped from planes in bombs or sacks which exploded 200 to 300 metres above the ground. Dozens of villagers were killed or have become ill as a result of them. The Vietnamese Embassy in London told *Nature* that the report was untrue and was an attempt to discredit the Vietnamese government "Only the US has used poison gas against the people of Indochina."

FOE takes action against "Tories cold war": Friends of the Earth in UK are launching a major campaign to force the UK government to supply adequate heating for its citizens this winter. Calling Britain's insulation standards a "national disgrace", FOE is critical of the lack of government incentives to provide insulation for houses and of the failure of the government to live up to its election promises. "It is ironic that in International Energy Conservation Month, the government is actually cutting its energy conservation programme while at the same time it is embarking on a potentially disastous major expansion of nuclear power." On 18 October a FOE team will instal insulation in a London home to make it "the best insulated flat in Britain" as a demonstration project to kick off the campaign.

"Don't worry, market forces will keep you warm!"

US firm cited for causing fertility hazards: The US Department of Labour's Occupational Safety and Health Administration last week cited the American Cyanamid Company for violating health standards by threatening the child-bearing capacity of female workers at a chemical factory in West Virginia. Last year the company admitted that it had refused to let women work in the plant's lead pigment section unless they had been sterilised, since it was concerned that the work might result in harming feotuses or affecting the women's fertility. The company says that it offered the women other jobs, although possibly at lower pay; however four women at the plant disclosed that they had been voluntarily sterilised in order to keep their jobs.

Announcing that a fine of $10,000 was to be levied on the company for violating health and safety standards by creating such working conditions, Dr Eula Bingham, head of OSHA, said that the agency was "breaking new ground" in its actions. "What this citation is saying is that companies are required to make the workplace safe for men and women. Part of making it safe is making it safe for reproductive capacity," Dr Bingham said.

The citation requires American Cyanamid to stop insisting that female workers working with highly toxic lead be sterilised.

Socialist countries discuss joint research: Representatives of the Academies of Science of the Comecon bloc met in Tallin (Estonian SSR) last month to discuss the main directions for joint research for 1981-85. According to the protocol of the meeting, joint research is to be extended in the fields of petrochemistry, quantum chemistry, mechanics, machine-design and technological processes. A longer-term draft cooperation programme in the natural sciences up to 1990 was also discussed. The meeting formally welcomed the Socialist Republic of Vietnam (represented by its National Centre of Scientific Research and Committee of Social Sciences) into the multilateral scientific cooperation agreement of the Academies of Sciences of Socialist Countries of 15 December, 1971.

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Holland's science shops for 'made-to-measure' research

Tony Ades reports on unorthodox efforts by radical Dutch scientists to match their work to the needs of society

SCIENCE shops in the Netherlands continue their steady growth and development. The annual report, to be released at the end of October, from the country's most established science shop at the University of Amsterdam, reflects an increasing scale of operations and plans for a deeper integration within the university structure.

Science shops (Wetenschapswinkels) are part of a movement that started in the early 1970s to provide a counterweight to government and industrial domination of science policy. Groups of radical scientists and students were then already organising themselves to distribute expertise and technical assistance to trade unions and other groups to whom it was not otherwise available. Mr F H P Trip, the Minister for Science Policy under the former liberal Labour government, had suggested that efforts of this sort should be supported as part of a broad attempt to democratise science.

A science shop does not undertake research itself. Its role is simply to put its clients, any group with a question, in touch with university staff able to do the research needed to answer it. The research itself is done on an unpaid and voluntary basis: faculty members must integrate it into their existing programmes, or students may undertake it as part of their dissertation. Science shops are therefore very cheap to run. Their costs need only cover advertising, copying, office expenses, and salaries for a small staff. The attraction is that the university can experiment with a novel relationship between the scientist and society, with minimal risk to the quality of education, and at negligible expense.

The movement gained impetus in early 1977 when the University of Amesterdam decided to reserve Dfl. 30,000 (£7,500) to start a science shop. The Secretary of State for Education in the present Conservative government cancelled this allocation, but the widespread protest that followed encouraged the university to take protective action: it established its science shop as an advisory committee to its governing board, a position where it remains virtually immune to political interference. The shop was duly opened in March last year, by the former minister Trip, and now has a budget of Dfl. 70,000, and a staff of four.

This year it fielded 550 enquiries, an increase of 50% on last year. Most came from unions (30%), environmentalist groups (20%) and neighbourhood organisations (15%); but a very broad range of clients was represented, including womens groups, welfare workers and individuals. 180 of these questions have been answered in the form of full research reports or written or oral advice. A further 100 were withdrawn or unsuitable, and the remaining projects are still under way.

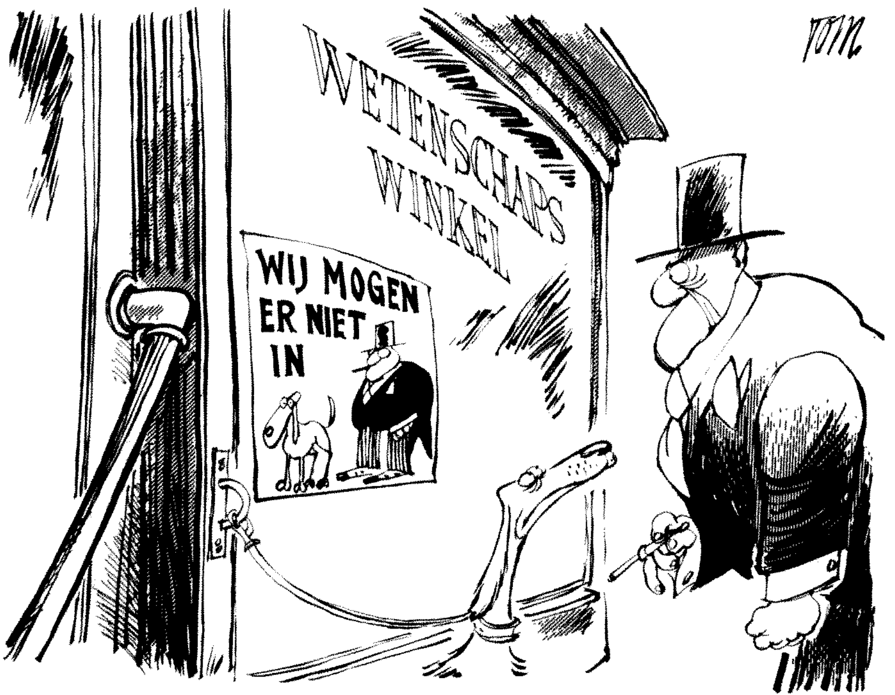
While it is the strongest, Amsterdam's shop was by no means the first. Science shops also operate from universities and technical colleges at Delft, Eindhoven, Groningen, Leiden, Maastricht, Nijmegen, Rotterdam, Tilburg, Utrecht and Wageningen. There is considerable variation in both the mode of affiliation to the parent university, and in the level of financial support. But support is increasing, and new shops are still being opened.

The most controversial aspect of the science shop movement is the criteria by which clients are selected. Any group or person may approach a shop, as long as they are unable to do the research themselves (or have it done elsewhere), have no financial interests in the results, and are in a position to use the results in a positive way. Because these criteria are explicitly intended to select for minority groups and exclude commercial interests, they have attracted the disapproval of the Minister of Education, Mr Arie Pais. In an open exchange with the University of Amsterdam in April this year, he agreed with the democratising role of science shops, but charged that the criteria threatened the "objective and unprejudiced" position of universities in society. Dr Loet Leydesdorff, chairman of the Amsterdam shop, answered that science shops were in fact furthering the university's public function by re-evaluating society's need for science, and orienting research to public ends. He added that there was nothing objective or unprejudiced about the government decisions that brought so many clients to the science shop door.

The matter is expected to remain at this impasse for some time. Science shops receive warm support from unions, students and the press; and while there is certainly ambivalence within some universities, the concept is so widely regarded as rational that strong opposition is seldom voiced in public.

The research questions that clients bring in are extraordinarily diverse, both in scale and in substance. Public and industrial health are perhaps foremost, and a number of universities have set up specialised chemistry shops to analyse potentially hazardous industrial by-products and check pollutant levels. Eindhoven's physics shop has dealt with a number of clients worried about traffic noise. In one project a speed meter was built so that a neighbourhood group could obtain accurate information on how fast cars were travelling down a particular street. In such cases, which are quite common, the science shop can be very effective in redressing imbalances in the availability of science. A local authority may drag its heals over vague protests about traffic or pollution, but it will give a serious hearing to citizens groups with hard data. Without science shops such data would often remain inaccessible.

In different universities there are shops specialising in architecture, agriculture and biology. In the latter field there are numerous enquiries into the effects on



"You can't come in here": money-making industrialists are banned from science shops

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animals and plants of waste dumping and weed killers, and on the environmental consequences of various farming practices.

Although science shops were originally oriented towards the exact sciences, and gained much of their early support in the health and pollution field, many of the questions now command attention from sociologists, economists and phychologists. In one recent incident, suspicions were aroused by a questionnaire that the Hilton Hotel planned to circulate among its staff. The union took advice from a sociologist and decided to boycott the questionnaire and design their own. There are numerous requests for research into the physical and mental health effects of projected highways, factory and housing developements. Second only to public health issues at Amsterdam are calls from labour unions who need to know about the likely effects of changes in management policy on conditons and employment levels in specific factories. Sometimes, prognoses on a company's balances are required.

Occasionally a science shop is called upon to provided a summary and inventory of research to date. Such requests have been received, for example, from a women's group concerned about 'the pill' and its side-effects. Often the client is simply directed to relevant literature, and the shop arranges for the material to be made available through the university library.

Some clients would exhaust the services of several think-tanks: on the books at Nijmegen are number of questions concerning energy. These include a comparative study of different energy sources, the general health risks of nuclear energy, calls for research into reactor design, how radiation norms are set, and the worldwide proliferation of nuclear facilities. Such issues should clearly be handled on a nationwide level: representatives of the different science shops meet once or twice a year, but their different internal structures and connections with their parent universities tend to inhibit coordination.

At the more light-hearted end of the scale was a question from a neighbourhood group: "Why do people like dogs? What are the psychological effects on the dog of living in a flat?" Another group, living near a milk factory, asked: "Is milk powder damaging to car-paint?" More ethereal but equally practical is the project suggested by a question put in by a political action group: "How can political action groups be most effective?"

While there is at present a mood of optimism and growth, the long term future of science shops is unclear. In Dutch universities research funds are distributed to sub-departments whose faculty plan their research programmes, with varying degrees of democracy, as a group. The group may earmark as much money as it wishes for socially-oriented research. Science shops will therefore survive as long as liberal scientists dominate the faculty groups. Indeed, at Amsterdam there are plans to close the gap between scientists and the public still further. Project centres are to be set up, each one responsible for a cluster of questions. As experience with clients' needs develops, the project centres will be able to formulate research priorities and feed socially oriented science policy back to the faculty groups.

But at some universities support for the science shop concept is much more tenuous. The shops depend on a small core of enthusiastic students and staff, operating in what they regard as a hostile environment. Many of the more radical proponents of science shops feel that this lack of institutionalisation is their best guarantee of a free hand, and are suspicious that the "professionalism" at Amsterdam is a capitulation. This is probably more a reflection of the different attitudes of the parent universities, than a real ideological split in the movement. The stress on working with rather than for the client, and the criteria by which clients are selected are strikingly consistent from one shop to another.

Yet there remain far more projects on the books than scientists willing to undertake them. It is widely agreed that the bottleneck arises from the clash between the career structure of the scientist and the interdisciplinary nature of the projects that clients bring in. The desire to do socially oriented research is quite strong: but so is the desire to have dissertations approved and papers published. the university scientist is under pressure to break new ground and to restrict research to the more tractable issues. Only the daring will tackle the open-ended questions asked on the other side of the science shop counter.□

Dutch minister warns of research cuts

Industry in the Netherlands is slowly cutting the money it spends on research and development, and concentrating on existing work rather than new developments. This warning was given by the new minister for science policy, Dr A. van Trier (above), in announcing his science budget for 1980. The 6.1 billion guilder budget is 417 million guilders up on this year's figure, but the bulk of the increase will be borne by the government. Industry's spending will go up by only 2.5% instead of the expected 9%.

The budget makes special allowance for industrial and technological research. Energy research will give a new importance to coal, which has been largely neglected since the introduction of natural gas led to the closure of Holland's mines in the 1960s. Now, plans to import foreign coal have brought it back into favour.

Dr van Trier proposed a new "minimum" programme for nuclear research, confining it to acquiring the technology necessary for the possible building of power plants and concentrating on safety, waste disposal and general health and environmental aspects. Only when an acceptable solution has been found for the problems of waste disposal, the safety of power plants and the risk of sabotage and terrorist action will three more nuclear power plants be built, Dr van Trier said. A decision in principle to go ahead with these was taken in 1974 but since then the argument about nuclear energy has intensified and the government now plans a broad and structured public debate, beginning next year. A decision about new power plants is not expected for two years. Research on fast breeders is to be reduced.

The science budget coincided with publication of a long-awaited energy memorandum which sets out the government's policy for the forthcoming debate. Conservation and diversification of resources are the two main features. Use of energy is expected to increase by between 52% and 86% by the year 2000 but the aim is to improve efficiency by 30% over the same period. Investment of up to 63 billion guilders will be necessary to reach this conservation goal, the memorandum says, to subsidise more and better insulation in houses and buildings and more efficient heating systems.

Alternatives like solar energy and wind power are expected to play only a minor part by the year 2000. The contribution of natural gas (now half of total use) is also likely to drop to 30% or less and will cost the consumer more. The government plans to be especially economical with the huge Groningen gasfield and switch slowly to other gas sources in the North Sea, Algeria and possibly Nigeria. The use of natural gas for electricity production will be phased out and replaced by oil and coal.

Casper Schuuring

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Séveso: the crucial question of reactor safety

A technical report on what caused Italy's dioxin disaster has too many loopholes, writes **Alastair Hay**

ITALIAN chemists and engineers investigating the causes of the trichlorophenol reactor accident in which the toxic chemical 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (dioxin) was discharged over the town of Séveso on 10 July 1976 have recently completed their report. Results of the investigation, carried out on behalf of the Italian Judiciary, form the first of several reports being prepared for the prosecution case against ICMESA, Givaudan and Roche, the owners of the trichlorophenol reactor. Other reports expected soon will cover the medical, agricultural, environmental and veterinary consequences of the accident.

The dioxin discharged contaminated a large area forcing over 700 people to be evacuated from their homes. Over 100 children in the area developed mild to severe forms of the disfiguring skin disease chloracne following contact with dioxin. And over 100 Séveso women are reputed to have had abortions — either in Italy or in other European countries — fearing the teratogenic properties of the contaminant; official Italian figures only refer to 34.

Many of the medical consequences of the Séveso accident are not subject to dispute. The long term effects, however, are still a matter of controversy owing to the recently confirmed carcinogenic properties of dioxin. But the subject which still generates real heat is the design and operation of the Séveso reactor. The latest Italian report will add more fuel to the controversy. But sadly, the report has some serious faults, chiefly because it makes claims without supporting evidence and accusations which are not borne out by further investigation. If used as the basis of the prosecution case against the reactor proprietors in its present form, much of it will not stand up to a rigid cross-examination.

The process to make 2, 4, 5-trichlorophenol involves the alkaline hydrolysis of tetrachlorobenzene with sodium hydroxide at a temperature of 170 — 180°C, in the presence of ethylene glycol as a solvent. In the course of the reaction, and particularly at elevated temperatures, two molecules of 2, 4, 5-sodium tricholorophenate condense to form 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin.

Dioxing formation is unavoidable during this process, but the quantity produced can be controlled by keeping down the temperature. Should the temperature rise and reach 230°C an exothermic reaction involving some of the reactor contents is initiated, heat is generated, the temperature rises rapidly and a considerable quantity of dioxin is formed. In a closed reaction vessel such as that used for trichlorophenol manufacture, the rising temperature leads to an increase in pressure and the eventual rupture of the reactor. At Séveso, the pressure blew the safety valve, venting the reactor contents over the residential area nearby.

It is the view of the report that the reactor was unsafe and the three companies involved are charged accordingly. The companies are: ICMESA — owners of the trichlorophenol reactor; Givaudan, ICMESA's parent company; and F. Hoffmann La Roche, of which Givaudan is a subsidiary. The report's clear intention is for all three to share the blame for what happened at Séveso, and the trio have never questioned this.

Also unlikely to be challenged is the report's statement that the condition of the reactor when it was closed down at the end of the run on 10 July 1976 was unusual and that it was the first time it had been left in that condition. Givaudan say this is true. But when confronted with this in court, the company is expected to argue that the reactor should never have been left as it was, but that water should have been added to the reactor after the run to cool the contents. The company also considers that it should have been informed by its subsidiary ICMESA, of any changes in operating procedure before they were introduced.

Solvent ratios — a dangerous deviation?

One of the more serious charges which Givaudan claims it can dismiss concerns the operating conditions of the reactor. The report says that the ratio between the solvent ethylene glycol and the starting product tetrachlorobenzene was too low, and this, it insists, was a "deviation from the process originally developed in [Givaudan's] patent USP 2,509,245, where the critical nature of this ratio was expressly mentioned". In the report's judgement the alteration in this ratio increased the risk of accident.

Givaudan admits that the molar ratio of the two solvents used at Séveso was 5.6 whereas the initial patent recommended a ratio of 8. However, the patent taken out in 1950 makes clear that this ratio was important only for the yield and quality of the trichlorophenol product.

The company's claim is surported by a recent review in the Italian journal *La Chimica el'Industria,* **61,** 108 (1979). Dr Giuseppe Ferraiolo of the University of Genova refers to the critical nature of the ratio between these two compounds. Mentioning various patents for the production of trichlorophenol Ferraiolo shows that only if the ratio is less than 5 is there a danger of an uncontrolled exothermic reaction occurring. Even at a ratio of 5 the reaction remains stable up to a temperature of 240°C.

Givaudan have confirmed they will contest one of the report's suggestions — concerning the relationship between the temperature reached by the Séveso reactor and the quantity of dioxin produced. In the conclusions of their report, the Italian chemists say that it is impossible to calculate the quantity of dioxin produced in the incident from the temperature of the reactor contents, and the concentration of dioxin in the residue left after the accident. No experimental evidence is advanced in the report to support this claim. Givaudan, on the other hand, claim that they have their own to refute it.

For the past three years the company has carried out numerous experiments in an attempt to recreate the conditions which precipitated the Séveso accident. So far this research has been unfruitful and the company is still unable to explain what happened at Séveso. However, what Givaudan does claim is that on the basis of its experiment it can predict how much dioxin is produced simply by knowing the temperature of the reactor contents. The company spokesman claimed that dioxin concentration increases with increasing temperature. This he says has already been reported (see *Nature* **273,** 582; 1978). From their evidence Givaudan claim that 530 g of dioxin was produced of which 240 g was ejected from the reactor. Givaudan calculated that the dioxin content of the reactor was 115 ppm; the Italian report says the measure value is 100 ppm.

Givaudan's experiments are to continue; some results, such as those concerning the nature and properties of the chlorinated phenols produced in trichlorophenol reactors, are expected to be published shortly. Asked to put a time limit on the experimental work Givaudan's spokesman was unable to do so. In the company's eyes, he admitted, this research might be considered "unproductive work". He still regretted, however, the fact that the company had not come up with any answers yet to explain the Séveso accident.

Much of the evidence in the medical and other spheres which will be presented in court aginst the reactor owners will be irrefutable. This, however, is unlikely to be the case where the operation and design of the reactor is concerned. Perusal of this first report of the Italian investigators suggests that they have not done enough homework, and that their prosecution case will be anything but convincing.

It is regrettable that the chemists have not focused more attention on inadequate safety devices for example the lack of a dump tank to contain any overspill from the reactor. The chemists would have been on surer ground here, for in spite of Givaudan's claims that the Séveso accident was 'unforeseen' it happened, and the consequences were appalling. ☐

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correspondence

Fast reactors do produce 60 times more energy than thermal reactors

SIR,—Despite Dr Jeffery's reference to "nuclear nonsense" (13 September, page 98), fast reactors do produce about 60 times more energy from the same quantity of uranium than do thermal reactors. The full reduction in uranium demand brought about by the introduction of fast reactors into a power system will not be achieved until all the thermal reactors have been replaced by fast reactors. The rate of replacement depends on many factors, including the particular rate of growth of the electricity demand, the breeding gain of the fast reactor, and the amount of plutonium needed for the initial charge of each reactor. For the particular parameters used in the calculations quoted by Dr Jeffery, uranium ore demand up to the year 2020 was reduced by a factor of 1.7, but he did not point out that some 10-15 years later there would be no need for any further uranium imports: a situation which would continue for centuries.

Dr Jeffery quotes calculations by Grainger and Merrick based on a 5.5% exponential growth rate. Such a high growth rate could not be sustained over a long period: it is about double the average growth rate over the last decade and would result by 2020 in a near 10-fold increase of the present installed generating capacity and a fuel consumption of 10^9 tonnes coal equivalent per year.

If a growth rate of much above 3% were to be maintained for one or two decades, two factors would become important. Firstly, reactor designs (which already exist) with better breeding performance than those currently specified, would be favoured. (Even the current designs have a better breeding performance than that assumed by Grainger and Merrick.) Secondly, the rate of introduction of fast reactors could be increased by fuelling some of the new reactors with U^{235} instead of plutonium. Even for a growth rate as high as 5.5% it would then still be possible to reduce uranium imports to zero within a few decades.

Yours faithfully,

B CUTTS
R D SMITH

UKAEA, Risley, UK

The National Computing Centre is not a quango

SIR,—I support the point made in your leader 'Hunting the Quangos' (2 August); Quasi Autonomous non-Governmental Organisations have undertaken many essential tasks in the public interest more effectively and for less cost than would have been the case if government departments had been used.

However, the inclusion of the National Computing Centre in your list of Quangos is incorrect. We could debate whether, or not, it ever was a Quango, depending on one's choice of definitions, but there is no doubt that today there is nothing 'Quasi' about the NCC's autonomous state.

In March 1978 an Extraordinary General Meeting changed the status of NCC from a grant-aided body to full independence. Monies received from government sources (about a quarter of the budgeted revenue of £4.4 million) are in payment for contracts or projects undertaken on behalf of government departments. A definition of Quangos on this basis would include many independent commercial or industrial organisations which undertake government contracts.

The only formal link with government in the present NCC constitution is the Secretary of State for Industry's power to recommend two directors (out of 19) and approve changes to the constitution. In no way does this influence the independence of NCC in its day to day activities, which, incidentally, include export earnings of nearly £300,000.

Yours faithfully,

D FIRNBERG

The National Computing Centre, Manchester, UK

Farm or laboratory for male chicks?

SIR,—Lord Halsbury considers the problems of animals on farms and in laboratories to be very different (16 August, page 534). There is however, an important case where the two are directly related. I refer to the neonate male chick (*Gallus gallus domesticus*) as an alternative to mammals for large-scale toxicity tests and other laboratory purposes. Farms in the United States alone have more than 275 million laying hens of strains developed to produce eggs at a minimum cost. Since the males of these strains have no commercial value (their meat is tough and stringy) they are destroyed after hatching. Thus, the males (cockerels) are a waste product, available in numbers exceeding 200 million per year in the United States alone. The chick offers advantages of low initial cost (approximately \$0.20 each compared with \$1.50 for mice and \$4.50 for rats) and low maintenance costs. Presumably, converting cockerels from a waste farm product to a source of income could also lead to lower prices for eggs.

There is ample precedent for laboratory uses of the chick, eg, Russell *et al.* (*Toxicol. Appl. Pharmacol.* 2, 558: 1960) compared the mouse and chick in toxicity determinations and Spooner and Winters (*Int. J. Neuropharmacol.* 5; 217; 1966) reported an extensive neuropharmacological profile of the chick. It would appear to be relatively staightforward for laboratories using large numbers of mammals to compare the chick with the mammalian model under their conditions. Similarities and differences will be found; each could prove useful.

A second suggestion for reducing the consumption of laboratory mammals is employment, where feasible, of the Dixon and Massey (*Introduction to Statistical Analysis*, McGraw-Hill, New York, 1957) "up-and-down" technique. This permits a 30-40% reduction in the number of animals required for an LD50 determination and is applicable to other tests, as well. A drawback which constrain applicability is that the effect upon a given animal must be known before the next animal can be tested.

While all living things should be protected from abuse, the Cruelty to Animals Act of 1876 recognised the differential attitudes of the public toward treatment of "cuddlesome pets" and of invertebrates. I suggest that resort to non-mammalian species, and to techniques which reduce the number of animals used in laboratories, might at least mitigate objection by the general public to the use of experimental animals.

Your faithfully,

ARTHUR CHERKIN

Sepulveda, California, US

Falsification of facts

SIR,—I have to disagree strongly with the letter by Lee Lorch (*Nature*, Vol 281, 13 September, page 98) on "Biased reporting of East European Science". My field of work is astronautics. In spite of all their activity, in this field and numerous international meetings, information from so-called Socialist bloc countries is nearly without value — ask any NASA international expert. There are some limited subject areas only (biomedical, and strictly scientific results), where my sweeping statement does not hold.

Any GDR citizen can visit the FRG, as far as the letter is concerned. It is the GDR which does not permit their citizens to travel freely. The accusation as stated by Larch is a gross falsification of the facts as they exist now since the shameful Berlin wall was built. Since this knowledge is widely available, I hold spreading of such disinformation to be irresponsible.

"Frustration and pessimism" is an exact description of the general mood in Soviet occupied Germany. I travel there quite frequently. Possibly Lorch does not speak German or succeeded in scaring his contact persons (easily done: they know the world they are condemnned to live in), so they were afraid of speaking freely.

Since I plan to continue travelling to East European countries, and to keep up the cordial personal relationship I enjoy with several scientists there, I ask that my name and affiliation be withheld.

ANON

Disillusionment with UK industry

SIR,—I wholeheartedly agree with the comments made in the article about Barry Francis and the engineering profession (9 August, page 442).

I became so disillusioned with British industry that I packed my bags and took my wife 4,000 miles west to the USA. Here I find that engineers enjoy a much better standing in society and those I encounter in industry hunger for the latest design and analysis techniques. Their appetite for using current research results contrasts so markedly with the conservative approach and outdated ideas so prevalent among British organisations. This is probably the reason why the UK iis termed Britain, rather than Great Britain. We no longer have the great engineers like Brunel, Watt and Stephenson and I don't see them materialising until many of the ideas indicated towards the end of the article are implemented.

Your faithfully,

BRIAN S THOMPSON

Wayne State University, Detroit, US

news and views

Direct imaging of atoms

from J. M. Thomas

EVER since Erwin Müller first demonstrated that individual atoms could be imaged (using field-ion microscopy, (FIM)) many scientists have endeavoured to view directly the atomic structure of molecules and crystals. The reasons for wishing to do so are numerous. First, some molecules, especially those involved in biochemical processes, are reluctant to crystallise, so that it becomes necessary to evolve methods for identifying and characterising certain segments, or the entire length, of isolated molecules. Second, crystalline solids are often composed of two or more finely intergrown phases and structures; and X rays and neutrons cannot solve the structure of such solids. Third, grossly defective (for example, highly non-stoichiometric or non-randomly stacked) solids defy structural elucidation by methods based on currently available techniques of X-ray crystallography. There is, therefore, exigent need for a tool that directly reveals the local rather than the averaged structure of solids. We should know more about dislocation cores, sub-grain boundaries and other extended defects, as well as interphase interfaces, if direct imaging of atoms were possible. Moreover, this achievement would have immense repercussions in metallurgy, medical and surface science as well as in solid-state physics and technology. Progress towards some hitherto inaccessible goals was reviewed at a recent symposium.*

Scanning transmission electron microscopy (STEM) is, in many respects, a natural complement to, and a direct descendent of FIM. Scattering of heavy atoms on light (carbon or boron) supports shows up readily when a minute pencil (<5 Å diameter) of electrons is scanned across a thin specimen; and there is no doubt from the work of A.V. Crewe (Enrico Fermi Institute, Chicago) and others that atomic resolution of isolated heavy atoms is readily possible. Unfortunately for biologically significant molecules the electron dose required to observe single heavy atoms (such as iodine, mercury or platinum which may be used to tag certain specific parts of a macromolecule) is at least an order of magnitude more than the dose which damages the macromolecule itself. Extensive beam damage to unstained molecules such as myokinase, valinomycin and vasopressin in STEM (F.P. Ottensmeyer, Ontario Cancer Institute) prompted A. Klug (MRC Laboratory of Molecular Biology, Cambridge) to ask whether the ashes of 'cremated' molecules are reminiscent of the original. As this question is still unresolved Klug argued cogently that biologists' time could be better spent trying to induce hitherto non-crystallisable macromolecules to form ordered two or three-dimensional aggregates so that they could be investigated by other methods.

For crystalline and defective solids STEM has two serious inadequacies: it is not easy, as pointed out by J.M. Cowley (Arizona State University), to orient specimens precisely, nor, at least on commercially available instruments, to record the diffraction pattern. These are essential prerequisites for high resolution structural imaging. It was, therefore, not surprising that most of the symposium was devoted to high resolution electron microscopy (HREM) by conventional (that is transmission) instruments.

V.E. Cosslett (University of Cambridge) described the performance of the high voltage (600 kV) high resolution microscope built as a collaborative venture between the Cavendish Laboratory and the Cambridge University Department of Engineering (W.C. Nixon). The minimum resolvable distance in a conventional electron microscope is proportional to $C_s^{1/4} \lambda^{3/4}$ so that improvements arise when the coefficient of spherical aberration (C_s) of the objective lens and the electron wave length, λ, are decreased (that is, accelerating voltage is increased). The strenuous attempts made to construct high-stability microscopes operating at 200, 600, 1,000 and 3,000 kV — all at present functioning in a few centres throughout the world — are therefore understandable. But how trustworthy is the projected image (assuming that the specimen suffers no appreciable beam damage or gives rise to minimal inelastic scattering)? When specimens are ultrathin, so that the weak phase object approximation holds true, interpretation is relatively straightforward, provided due regard is paid to the contrast transfer function of the objective lens. With thicker specimens multiple electron scattering is extensive and the observed image may bear no resemblance to the projected structural features of the object. Fortunately, a powerful procedure devised by Moodie (CSIRO, Melbourne) and Cowley and known as the 'multislice' method, enables the electron microscopic image — and its dependence on specimen thickness and the extent of lens defocus — to be calculated with considerable confidence. This method constitutes a means of matching computed with observed images. If the agreement is satisfactory, credence is given to the assumed structure that was fed into the calculation. The overall procedure of structure determination by electron microscopy is, therefore, akin to the trial and error method extensively adopted by X-ray crystallographers before the advent of direct methods. This restrictive state of affairs does not engender complacency, and is one of the reasons why theorists such as Moodie have developed a way, which he outlined, of achieving, under specific experimental conditions, direct inversion from the back focal plane.

L.A. Bursill (University of Melbourne) described how, by judicious use of 100-kV microscopes, structural resolution of less than 3.0 Å can be achieved. He demonstrated how novel features in certain naturally-occurring tunnel structures and incipient ferroelectrics are revealed by microscopic investigations. He also examined the question of 'model sensitiveness' of the image match in relation to the structure of the rather

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*Nobel Symposium 47 on 'Direct Imaging of Atoms in Crystals and Molecules', was organised by P. Kierkegaard, A. Magneli, L. Kihlborg and M. Lundberg, and held at the IBM Nordic Education Centre, Lidingö, Sweden in August, 1979. The proceedings are to appear in a forthcoming issue of *Chemica Scripta* and also in a special publication edited by L. Kihlborg.

J.M. Thomas is Head of the Department of Physical Chemistry, University of Cambridge.

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enigmatic platelets known to occur (on {100} planes) in diamonds.

The trial and error method for determining crystal structures by HREM was strikingly illustrated by N. Uyeda (Institute for Chemical Research, Kyoto University) for a new phase of V_2O_5; by M. Sundberg (Arrhenius Laboratory, Stockholm) for a new tetragonal tungsten bronze consisting of pentagonal columns in a composition $W_{24}O_{68}$; by L. Kihlborg (Arrhenius Laboratory) for a fascinating range of intergrowth tungsten bronzes ($M_x WO_3$ with M = K, Rb, Cs and so on, and $X \leqslant 0.10$) consisting of hexagonal tunnels; and by S. Horiuchi (National Institute for Research in Inorganic Materials, Japan) on a new derivative of silicon nitride ($Si_3N_4.Y_2O_3$). None of these structures could have been solved by X-ray methods.

The image matching method was shown by J.M. Thomas (University of Cambridge) to be capable of distinguishing which of two possible new chain structures in silicate minerals exists as an extended, two- or three-dimensional defect inside nephrite jade, work which illustrates the power of the multislice method. A. Bourret (Centre d'etudes Nucléaires de Grenoble) likewise showed how structural details of one-dimensional faults in elemental semiconductors (Si and Ge) could be clarified by image matching; and S. Ijima (Arizona State University) has put the image matching approach to good use in his studies of the layer by layer build-up of graphitic carbon sheets. Even when no multislice calculations are carried out, structural insight may sometimes be gained by careful comparison of observed and 'expected' images, a fact vindicated by the work of O. Krivanek (University of California, Berkeley) and J.L. Hutchison (University of Oxford). The former, working with the Kyoto 500-kV microscope, showed that, as had been surmised, sub-grain boundaries in the Ge are made up of 5-and 7-fold rings: the latter has succeeded in clarifying the structure of both polytypic variants in certain perovskites and of Aurivillius phases. Horiuchi presented convincing proof that 1,000-kV high resolution images of $Nb_{12}O_{29}$ (a block structure) surpassed those taken with the best commercially available 100-kV microscopes. He also demonstrated that, by imaging ferroelectric crystals of $Bi_7Ti_4NbO_{21}$ along two or more high symmetry directions, the point group and hence the real space structure, may be determined directly. Resolution is sufficient to indicate, on the microscopic image, that the heavy atoms in the Aurivillius phase $Bi_2W_2O_9$ are not arranged on undistorted cubic lattice.

Two other studies illustrated the importance of high voltage HREM. N. Uyeda (Kyoto University) showed superb micrographs of hexadecachlorocopperphthalocyanine, which by adroit sample preparation (Uyeda *et al. J. appl. Phys.* **43**, 5181; 1972) will form pillars of overlapping molecules running essentially in the direction of projection. Apart from the central Cu and the peripheral Cl atoms, the outlines of the porphyrin rings, in this exceptionally beam-stable organometallic material, may be satisfactorily imaged. Thomas, with the aid of micrographs taken on the Cambridge 500-kV instrument (Jefferson *et al. Nature* **281**, 51; 1979) demonstrated how a point-to-point resolution of about 2.7 Å may now be comfortably achieved, thus making it possible to identify corner-sharing SiO_4 tetrahedra, and hence the backbone repeat patterns of the pyroxenoid minerals wollastonite and rhodonite.

It is most unlikely that solid state reactions, other than the kind described by R.J.D. Tilley (University of Bradford) who discussed the way in which electron irradiation generates so-called crystallographic shear (CS) planes in WO_3 when the latter, through oxygen loss, becomes progressively non-stoichiometric, will prove generally amenable to direct, *in situ*, investigation by HREM. In the words of J.S. Anderson (University College of Wales, Aberystwyth) the absence of 'eye-witness' procedures compels us to rely on the coroner's report in reconstructing the scene of the crime. With the aid of micrographs taken by his collaborator Sian Crawford Davies he gave a fascinating account of the quasi-homogenous oxidation reactions that take place within solids derived from the archetypal block structure Nb_2O_5, allegedly "the most flexible material in the whole of inorganic chemistry". Electron diffraction patterns of reactant and products in these systems are sometimes indistinguishable; but high resolution images enable one to chart (post event) the series of subtle, unit cooperative jumps (of the kind strikingly illustrated in other systems in a time-lapse movie film shown by H. Hashimoto, Osaka University) that may occur along a favoured line of atoms within these remarkably compliant materials. HREM studies clearly permit a 'projected' radial distribution pattern surrounding the locus of chemical reaction to be constructed in real space, from 'samples' consisting merely of a few tens of atoms. Post event rationalisation by J.V. Sanders (CSIRO, Melbourne) guided by HREM studies of hydrodesulphurisation catalyst particles has brought to light several unexpected facts, including the occurrence of encapsulating layers (a few atoms thick) of MoS_2. Comparable approaches have been adopted by Krivanek on silicon-silica interface. A. Howie (Cavendish Laboratory, Cambridge) dealt with amorphous materials, the radial distribution functions of which (extracted from X-ray data) do not adequately elucidate the local structure. Howie pointed out that HREM also runs into serious difficulty here, largely because of the problems stemming from the inherent unreliability of the projection procedure. Nevertheless, valuable progress has been made and Howie concentrated on how various experimental and processing (phase randomisation) approaches have been of value in the study of 'amorphous' metals, silicon and carbon.

Intermetallic systems were discussed from two viewpoints. S. Amelinckx (SCK/CEN, Mol), in an extensive series of unusual examples (dealing with alloys such as Au_4Mn, Au_5Mn, $Au_{11}Mn_4$) demonstrated how HREM, supplemented by selected area diffraction has greatly enlarged our knowledge of imperfectly ordered alloys. He emphasised how, under certain circumstances, there can be advantages in utilising superlattice spots to form relatively low power images rather than to push resolution to its limit (by taking the 'fundamental' diffraction spots). L. Stenberg's discussion (based on his work with S. Anderson) of some tetrahedrally close-packed alloys (such as Mo_3CoSi, Zr_4Al_3 and $MgCu_2$) strongly suggested that, as a result of advances in HREM, the structural inorganic chemist and the metallurgist will soon be brought closer. The concepts of CS, anti-phase boundaries and chemical twinning, which have proved valuable in interrelating structures among binary and ternary oxides seem to be equally useful in discussing these close-packed alloys. □

From ligands to legumes

from A. W. B. Johnston

THE interaction of atmospheric nitrogen with synthetic catalysts, and the interaction of *Rhizobium* with clover are two distantly related areas of research united by the phenomenon of biological nitrogen fixation. These two topics have been studied in depth by two eminent workers, Professor J. Chatt (Director of the Unit of Nitrogen Fixation, Sussex) and Dr P. S. Nutman (Head of the Soil Microbiology Department, Rothamsted) respectively, and to mark their impending retirements the Phytochemical Society of Europe organised a symposium* which included contributions on the whole spectrum of current nitrogen fixation research.

At the heart of the nitrogen fixation process lies the enzyme nitrogenase. W. H. Orme-Johnson (University of Wisconsin, Madison) considered the arrangement of the two metals, Fe and Mo, which are present in the protein subunit that is responsible for the binding and reduction of nitrogen. The Mo is present in a metal cluster that also contains Fe (MoFe

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*An International Symposium on Nitrogen Fixation was held at the University of Sussex on 17-19 September, 1979, sponsored by the Phytochemical Society of Europe.

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cofactor); the majority of the Fe is present in Fe—S clusters that are in a highly reduced state and which are in a less exposed position than is the MoFe cofactor.

A point made by both J. Chatt and R. H. Burris (University of Wisconsin, Madison) was that nitrogenase has different affinities for the substrates N_2, H_2 and C_2H_2 depending on its oxidation state. Since the reduction of C_2H_2 to C_2H_4 is often used to assay nitrogenase activity it follows that it is not straightforward to extrapolate the values obtained from this assay to calculate absolute amounts of nitrogen that are being fixed.

Burris also referred to the fact that in *Rhodospirillum* and in *Azospirillum* the success in isolating the Fe-containing nitrogenase component is variable in different laboratories. It seems that there is an inactive form of the protein which can be activated by enzymatic removal of moieties (possibly adenine) and that the occurrence of the active or inactive forms depends upon the growth medium.

Nitrogenase needs a source of energy and electrons for reducing power. H. Haaker (Agricultural University, Wageningen) proposed that in *Azotobacter vinelandii* and in the bacteroids of *Rhizobium leguminosarum* the transport of electrons to nitrogenase depends upon the electrical component ($\Delta\psi$) of the proton motive force since there was a correlation between the value of $\Delta\psi$ and nitrogenase activity. In free-living nitrogen fixing bacteria, NH_4^+ is a potent repressor of nitrogenase but in the case of *Rhizobium* bacteroids, nitrogenase is relatively immune to NH_4^+ repression. Interestingly, the addition of NH_4^+ to *A. vinelandii* caused a drop in $\Delta\psi$ but the $\Delta\psi$ of *Rhizobium* bacteroids was immune to the addition of NH_4^+.

There are some bacteria that are doubly blessed in that they can fix nitrogen and can obtain the energy for fixation by photosynthesis. J. R. Gallon (University College of Swansea) considered whether in the case of blue green algae, photosynthesis could directly provide ATP and reductant to nitrogenase. Some evidence to support this came from the finding that inhibition of photosystem II rapidly inhibited nitrogenase activity in *Gloeocapsa*. However nitrogenase activity was quickly restored and it was suggested that this recovery was due to the switch on by another, non-photosynthetic, source of energy.

Bacteria that are fixing nitrogen liberate gaseous H_2 due to the reduction of protons by nitrogenase. Many species possess a hydrogenase that can be used to recoup some of the energy that would otherwise be lost. H. J. Evans (Oregon State University) discussed the hydrogenase system in *Rhizobium japonicum* in which the enzyme is sufficiently active to allow autotrophic growth on hydrogen as sole energy source. The fact that when hydrogenase-deficient mutants were used to inoculate soybeans the yields were about 30% less than when wild-type strains were used shows that the hydrogenase system is of real agronomic importance.

The developments in the genetics of nitrogen fixation were discussed by a number of speakers. The most detailed analyses have been on the *nif* gene cluster of *Klebsiella pneumoniae* and these were

Squirrels kill kids but are nice to relatives

from John Krebs

BELDING's Ground Squirrel (*Spermophilus beldingi*) conceals a vicious habit beneath its charming, furry coat. P. W. Sherman has studied a population of 250-300 of these little beasts high up in the Sierra Mountains of California since 1974, and he reported on some of his results at the XVI Ethological Congress.* The squirrels hibernate underground beneath the snow for about 8 months of the year and have a brief reproductive season during the months of May-August. Their life is a tough one as might be expected from such an extreme environment: 54-93% of juveniles and 23-68% of adults die during winter hibernation, and the survivors have to face the dangers of numerous nocturnal (coyotes, and bears) and diurnal (raptorial birds, weasels and coyotes) predators, as well as the risk of being run over by a passing car. These kinds of natural hazards are well known as causes of death in natural populations of many species, but more unusual is the fact that a considerable number of young squirrels die as a result of infanticide. About 8% of all young are dragged from their burrows by adult squirrels and killed before their mothers can come to the rescue. Sherman's detailed analyses of the causes and consequences of infanticide lead him to the following conclusions.

Adult females and yearling males are the commonest killers. Adult females kill when their own young have been destroyed by predators: the bereaved mother moves to a new place (choosing carefully to go to a place less susceptible to predator attack) and tries to kill the young of a mother in the area. If she is successful in killing young the female settles near the burrow of her victims. It seems, therefore, that infanticide by adult females is a form of competition: the female removes potential future competitors by disposing of her new neighbour's young. The young male killers, unlike the adult females, eat their victims, and Sherman suggests that the benefit derived is simply that baby ground squirrel is a good source of protein for a growing lad. A young male's future survival and reproductive success in this highly polygynous species depends heavily on fighting ability and hence body size (Sherman in *Natural selection and social behaviour: recent research and new theory.* (Alexander & Tinkle eds.) Chiron Press 1979).

Thus infanticide is far from being a pathological behaviour shown by a few squirrels under abnormal conditions; it is a normal part of the day-to-day business of competition and survival. Female squirrels with young are highly territorial during the period in which their infants are helpless to defend themselves, and mothers with large territories have the lowest risk of infanticide, which suggests that territory defence might be an anti-infanticide device.

Belding's Ground Squirrels are not, however, universally nasty: close relatives are nice to each other. The squirrel society is matrilineal. Females pass on bits of their defended home area to daughters while males disperse well away from home soon after weaning. Sherman's accurate data on kinship enabled him to show that close female relatives cooperate with each other in a number of ways. They share parts of their territories, do not attack each other very often, and help each other in guarding their burrows against infanticidal intruders. Mothers also put themselves at a demonstrable risk when a predator approaches by giving alarm calls to warn close relatives of the danger (Sherman *Science* **197**, 1246; 1977). Squirrels without close relatives in the vicinity (males and transient females) do not give alarm calls, which is consistent with the fact that they do not stand to make a genetic profit by self-sacrifice.

Sherman's unique and impressive data show that altruism (or nepotism as he prefers to call it) occurs between close relatives. This is to be expected since an altruist's genes are propagated not just through children but also through other relatives, and some would argue that Sherman's data are a good test of the theory of kin selection. In a sense, however, the theory is not testable. It is a logical generalisation of the theory of Natural Selection and is not one of a number of alternative, refutable hypotheses. However, an example of the kind of interesting question which arises from kin selection theory is Sherman's observation (*Nat. Hist.* **88**, 80; 1979) that while mothers, daughters and sisters show nepotism towards one another, more distant relatives such as aunts and nieces or grandmothers and granddaughters do not. As Sherman puts it, the reasons for this limit to nepotism remain an enigma.

John Krebs is a Lecturer in Zoology in the Edward Grey Institute of Field Ornithology, University of Oxford.

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*The XVI International Ethological Congress was held in Vancouver, BC, Canada, 18-27 August, 1979.

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considered by M. J. Merrick (Unit of Nitrogen Fixation, Sussex). Fourteen genes have been identified and these are organised into eight transcriptional units. A useful tool in the study of gene control in this system involves the insertion into the different *nif* operons of a derivative of bacteriophage Mu carrying the structural genes but not the promoter of the *Escherichia coli lac* operon. Thus expression of any *nif* transcriptional unit can be determined by assaying β-galactosidase production. By such means it was shown that when *K. pneumoniae* was derepressed for *nif* expression different *nif* genes were turned on at different times. The allocation of particular proteins (seen on two-dimensional gels) to particular *nif* genes was demonstrated in posters by J. Houmard (Institut Pasteur) and by W. Klipp (University of Erlangen). Klipp also showed that the DNA corresponding to the *K. pneumoniae nif* region was significantly more dense than the typical value for the chromosome of this species and is also flanked by two sets of inverted repeats. It was suggested that the *nif* genes may have been acquired relatively recently by recombination with some other bacteria.

The close relationship of *nif* DNA from different nitrogen fixing species in widely separated taxonomic groups was in fact demonstrated by F. Ausubel (Harvard University). A segment of the *nif* operon containing *K. pneumoniae* structural genes for nitrogenase has been cloned and been found to hybridise with DNA from 8 different nitrogen fixing species but not to 12 other, non-fixing species. In the case of *R. meliloti,* hybridisation was specifically with plasmid DNA, a conclusion also supported by data presented by A. Kondorosi (Hungarian Academy of Sciences, Szeged). J. E. Beringer (John Innes Institute, Norwich) reported that in *R. leguminosarum,* some of the genes that are required for nodulation and for fixation within the nodules have been located on the same resident plasmid found in a strain of this species.

The past 10 years have seen a great rise in interest in biological nitrogen fixation, stemming largely from the awareness that the production of commercial nitrogenous fertiliser makes significant demands on fossil fuel energy. This interest has been reflected in the real developments that have been made in many areas of research in this field and these were amply demonstrated in many of the contributions that were given at the symposium. ☐

A. W. B. Johnston is at the John Innes Institute, Norwich.

How do toxins penetrate cells?

from R. C. Hughes

THE death of the Bulgarian broadcaster, Georgi Markov, apparently by a ricin bullet, received considerable press coverage recently. In less sombre vein the cytopathological effects of ricin and other toxins are of wide interest since they hold out some hope for site-directed killing of tumours and may also throw some light on the mechanism of action and entry into cells of molecules such as hormones and growth factors.

At a recent workshop meeting*, F. Stirpe (Instituto di Patologia Generale, Bologna, Italy) described the effects of injection of ricin into rats as severe necrosis of sinusoidal (Kupffer) cells of the liver and of the red pulp of the spleen. Abrin and modeccin are related toxins, binding to similar glycoprotein receptors as ricin. While modeccin affects the liver, abrin specifically brings about severe necrosis of acinar pancreatic cells. Like many toxins, ricin, abrin and modeccin bind to cell surface recptors, enter the cell and exert a cytoplasmic effect, in these particular cases by inhibition of protein synthesis. All three toxins apparently block protein synthesis at an identical site on 60 S ribosomes and are structurally very similar, consisting of two polypeptide subunits. One subunit (B chain) binds to cell surface galactose residues following which the toxic A subunit is taken up into the cell and transported to the ribosomes (see Olsnes in *Transport of Molecules in Cellular Systems* (ed. Silverstein) Dahlem Conference, Berlin, 103;1978). Since the A subunits of ricin, abrin and modeccin are biologically indistinguishable the interesting target specificity described by Stirpe must be due either to small differences in the preferred surface receptors for each toxin expressed on different cell types or to differences in the steps subsequent to binding. Stirpe suggested that these steps may include processing or degradation of the toxins, and such processing may be essential in mediating cytotoxicity or if carried to excess, may destroy biological activity.

The mechanisms by which bifunctional molecules such as ricin traverse the cell surface membrane and transport the toxin portion of the molecule to the cytoplasmic site of action are poorly understood. A. M. Pappenheimer (Harvard University) presented compelling evidence for the direct transfer of the toxic principle of diphtheria toxin. Diphtheria toxin (a 62K protein) contains hydrophobic peptide sequences in the B region (40K), located at the C-terminal of the whole toxin molecule (P. Falmagne, Université d'Etat à Mons, Belgium). Presumably diphtheria toxin first binds to surface receptors (as yet unidentified) and then inserts into the phospholipid bilayer by more stable transverse associations mediated through the hydrophobic peptide sequences (see Boquet *et al. Proc. natn Acad. Sci. U.S.A.* **73,** 4449; 1974). Pappenheimer believes that such an association requires the cleavage of a C-terminal sequence of the B fragment which possibly is facilitated by the unusually high incidence of basic residues in this region. Additional features of diphtheria toxin action on sensitive cells comes from study of mutant proteins (Pappenheimer). Toxin (CRM 45) of abnormally low molecular weight (45K) isolated from a chain-terminating mutant contains an enzymically active A fragment (22K) but lacks much of the B region sequence required for interaction with surface receptors. CRM 45 toxin contains the hydrophobic sequence, however, and is about 1-2% as toxic as normal toxin when injected into chickens and is equally toxic when injected into the brain of mice where the cells chiefly affected are Schwann cells. Fragment A itself, lacking the hydrophobic sequence of CRM 45, is at least 100-fold less toxic in this system emphasising the importance of the hydrophobic sequence in mediating toxicity. Pappenheimer suggests that the intact diphtheria toxin, the CRM 45 protein and fragment A are taken up into cells by different mechanisms of steeply decreasing efficiency. Evidently even the latter inefficient uptake is sufficient to kill a cell because it had been calculated that one or two toxin molecules in the cytoplasm are sufficient.

This high sensitivity probably also explains the recent success in synthesis of hybrid toxic molecules. Despite one negative result of Chang *et al.* (*J. biol. Chem.* **252,** 1515; 1977) who found no killing of mammary cells by a diphtheria A subunit — human placental lactogen complex, more recent reports have shown that diphtheria A subunit coupled with concanavalin A (Gilliland *et al. Proc. natn Acad. Sci. U.S.A.* **75,** 5319; 1978), *Wisteria floribunda* lectin (Uchida *et al. J. biol. Chem.* **253,** 6307; 1978), and antilymphocyte globulin (Thorpe *et al. Nature* **271,** 752; 1978) or ricin A subunit coupled with human gonadotropin (Oltmann & Heath *J. biol. Chem.* **254,** 1022; 1979) or concanavalin A (Yamaguchi *et al. J. natn Cancer Inst.* **63,** 1387; 1979) are cytotoxic, although at high doses and over rather long periods. Presumably, the processing of the conjugates is mediated after binding of the surface binding ligands and leads to penetration of the toxic A subunits. It is not clear whether this uptake

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R. C. Hughes is at the National Institute for Medical Research, Mill Hill, London.

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*The Workshop 'Toxins with intracellular targets' was organised by S. Olsnes (Norsk Hydro's Institute for Cancer Research, Oslo) and P. Boquet (Pasteur Institute, Paris) as part of the 6th International Symposium on Animal, Plant and Microbial Toxins held on 19-24 August, in Uppsala, Sweden.

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is similar to that described for the intact diphtheria toxin or is more closely analogous to the less efficient mechanisms by which CRM 45 or diphtheria fragment A penetrate the cell.

The uptake of ricin into cells may proceed in a way analogous to the Pappenheimer model for diphtheria toxin. Ricin undergoes a conformational change after binding to oligosaccharide receptors that may facilitate direct transfer, for example by exposure of hydrophobic segments. S. Olsnes (Norsk-Hydro's Institute for Cancer Research, Oslo) and R. C. Hughes (National Institute for Medical Research, Mill Hill) showed that ricin action is inhibited at low temperatures. Mosquito cells and baby hamster kidney cells are protected significantly from ricin toxicity at 28°C although binding to cells and inhibition of protein synthesis in cell free systems are as effective at 28 as at 37°C (T. Butters & Hughes). Since mosquito cells grow optimally at 28°C the temperature effect is likely to be attributed to the ricin molecule itself, possibly in the conformational change mentioned above. One cold sensitive structure could be eliminated as contributing to the temperature effect. Colchicine, which disrupts cytoplasmic microtubules in hamster fibroblasts, has no significant 28°C effect on ricin toxicity. Cytochalasin B does protect cells after prolonged treatment before the challenge with ricin suggesting a possible involvement of microfilaments in ricin penetration into cells.

Bundles of microfilaments directly under the surface membrane are believed to have a role in receptor-mediated pinocytosis by contraction of the plasma membrane containing the surface absorbed ligand. However, the role of endocytosis in mediating entry of toxins is still controversial. Receptor-mediated endocytosis of toxins has been observed directly in several systems but such experiments use very high levels of toxins for visualisation, usually much higher than the cytotoxic dose. It is then not clear whether the toxin molecules seen enclosed in endocytotic vesicles within the cytoplasm are those responsible for the toxic effect by leakage of toxin from just one vesicle. Endocytosis is clearly involved in uptake of lipoproteins into cells at the specialised surface sites, enriched in the specific receptors, known as 'coated pits' (Goldstein *et al. Nature* **279**, 679; 1979). However, lipoprotein uptake is geared to the lysosomal degradation of proteins and release of cholesterol and it would be premature to assume similar mechanisms operating in toxin action.

Nonetheless, J. L. Middlebrook (US Army Medical Research Institute of Infectious Diseases, Fort Detrick) showed that both diphtheria toxin and *Pseudomonas* exotoxin A may enter some cells by receptor-mediated endocytosis. Local anaesthetics and lysosomotropic agents such as choloroquine protect cells from toxicity. Similarly, G. T. Keusch (Tufts University, Boston) showed that lysosomal stabilisers or drugs affecting microfilament or microtubule function inhibited the effect of *Shigella* exotoxin on sensitive cells while DEAE-dextran, a stimulator of endocytosis, significantly increased the cytotoxic response of the cells to the toxin. Keusch proposed that a limited degradation of *Shigella* exotoxin in the lysosomes is a necessary step in cytotoxic action, which is reminiscent of the proposals for ricin by Stirpe and for diphtheria toxin by Pappenheimer. Such processing seems to be a common feature of many models of toxin action. Clearly, lysosomes are specialised for such protein degradation but specific limited proteolysis at cell surfaces is still an attractive alternative.

The evidence at present suggests strongly that toxin action mimics the normal mechanisms of action of hormones and growth factors. In some cases similar or identical surface receptors are involved and it is quite likely that the subsequent steps in mediating these diverse biological effects may turn out to be similar. ☐

Meteoritics — life begins at seventy

from A.E. Fallick and C.T. Pillinger

IT is now known that nearly 20% of the naturally occurring elements have isotopic variations which are not the result of nuclear reactions within the Solar System, and therefore it was something of a novelty for the recent meeting of the Meteoritical Society in Heidelberg* to close without a report of a new isotope anomaly. However, interest in the subject is not declining. In fact the opposite is true, and accordingly two reviews were devoted to this exciting area, G.W. Lugmair (San Diego) dealing with the state of the art and W. Hillebrandt (Munich) considering the challenge presented to theories of nucleosynthesis.

The group led by Peter Eberhardt (Physikalisches Institut, Berne) has perhaps best demonstrated the painstaking approach now needed to track down the location of anomalies. Since 1974 various members of this department have been investigating the occurrence of a component of the rare gas neon (called neon-E) in a number of meteorites. After an extremely detailed study starting from 6 g of the Orgueil carbonaceous chondrite, M.H.A. Jungck and Eberhardt have isolated a few hundred micrograms of an unidentified, but probably carbonaceous, component which on stepwise heating releases neon having an isotopic composition $^{20}Ne/^{22}Ne = 0.008$ and $^{21}Ne/^{22}Ne = 0.0006$, which means that it is practically pure ^{22}Ne. One possibility is that neon-E is the daughter product of ^{22}Na, an isotope with a 2.6 year half-life.

Much interest is still focused on the Allende meteorite. For example, E.K. Jessberger and E. Dominik (*Nature* **277**, 554; 1979) recently reported a potassium-argon age of 5.08×10^9 year for an Allende inclusion. Isotopic measurements on potassium by W. Stegmann and F. Begemann (Mainz) have shown a composition within ½% of the terrestrial value, which discounts the possibility that the extraordinarily high age is an artefact explicable in terms of an enhancement in ^{40}K.

Isotopic anomalies are certainly no longer confined to meteorites. Various workers, including J.F. Kerridge (Los Angeles) and R.N. Clayton (Chicago), have for some time been advocating a secular change in the nitrogen isotopic composition of the solar wind. M.H. Thiemens and Clayton now report a new low δ value of −110‰ (relative to atmospheric gas) for nitrogen in an Apollo 17 lunar soil. Whilst astrophysicists are presumably aghast at having to explain nitrogen isotopic fluctuations of 30% in the source region of the Sun, Clayton admits that the onus is on the experimenter to verify conclusively the data.

Also on the Moon, an examination of the siderophile trace element pattern of Apollo 17 black glass spheres by J.W. Morgan (Reston) suggests that these glasses are not purely of volcanic origin, as has been proposed. The relative abundance of seven out of eight elements points to the possibility that a particular type of iron meteorite splashing into a lava lake could have been partly responsible. Speaking from the floor, Ed Anders (Chicago) recalled that when such an origin was proposed a number of years ago there was a degree of incredulity amongst the audience, but he felt the evidence now presented had reopened the debate.

Inevitably a number of 'old faithfuls' reared their heads. Although first recognised in 1802 and named in 1863, chondrules remain an enigma. Klaus Keil (Albuquerque) eloquently dismissed as many origins for these as there are students of the subject minus one — and in as many seconds — during his review of the field. On the basis of textural and chemical evidence, and laser-induced melting simulations, he favours a secondary impact origin rather than some primary process. New food for thought for chondrule students, however, came from Kurt Fredriksson (Smithsonian Institute) who, because of improved techniques in manufacturing ultrathin polished sections, was able to observe a carbonate inclusion in the centre of a chondrule from the Allegan meteorite.

In the new areas of endeavour it was good to see a number of papers devoted to

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*The 42nd annual meeting of the Meteoritical Society was held at Heidelberg on 3–7 September.

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recently discovered Antarctic meteorites. One interesting point made by L. Schultz (Mainz-Paris consortium) was that a sample collected in the Allan Hills must have had a preatmospheric radius of only 1-2 cm. It was previously thought that such small samples did not survive passage to Earth. At the other extreme several investigators, including members of a Chinese delegation, described work on the Kirin meteorite, a four-tonne chondrite which fell recently in the People's Republic. A survey of the ancient literature of China by Tu Guangzhi (Institute of Geochemistry, Guiyang) reveals many credible records of phenomena attributable to falling meteorites, the earliest being in 2133 BC. We hope that China's future participation in meteoritics will be as prolific as its past.

Another hope for the future is the planned chemical investigation of cometary dust particles during possible fly-by of Halley's comet and rendezvous with Temple 2. B.-K. Dalmann (Max Planck Institut für Kernphysik, Heidelberg) described various experiments for the secondary ion mass spectrometer on the rendezvous mission, including an attempt to measure stable carbon isotope ratios. Another possible source of cometary debris for analysis, suggested by Dave Brownlee (University of Washington, Seattle), is the collection of micron-sized particles from the stratosphere by U2 aircraft. Perhaps study of these grains could reveal the carbon isotopic ratio of comets if a sufficiently sensitive technique were available.

Finally as an encouragement to those of us who have yet to make a significant contribution to science, the Meteoritical Society chose to honour Paul Ramdohr (Max-Planck-Institut für Kernphysik, Heidelberg) with its Leonard Medal. Although Professor Ramdohr claims an interest in meteoritics from his first months as an undergraduate in 1909, he did not commence the work for which the medal was awarded until after his 'retirement' in 1960 at the age of seventy! □

A.E. Fallick and C.T. Pillinger are at the Department of Mineralogy and Petrology, Cambridge.

Is there anyone out there?

from J. Tarter and B. Zuckerman

"WHERE are they?" This question attributed to Enrico Fermi held centre stage during a full day symposium on 'Strategies for the Search For Life in the Universe' at the XVII General Assembly of the International Astronomical Union.

The first topic under discussion was 'N' the number of communicative civilisations that currently exist in our Milky Way Galaxy. I. Shklovsky (Sternberg Astronomical Institute) suggested that the choice of a value for N may be a very large number ($\geqslant 10^9$). Considerably less optimistic was M. Hart of Trinity University who argued that N is likely to be very small (~ 1). Hart pointed out that a calculation of N by means of the so-called Drake equation is essentially impossible owing to the orders of magnitude uncertainty in many of the terms. A better way to estimate N, according to Hart, is to assume some value for N and enquire about its effect on observables. In particular, he asked, if N is very large then, "where are they?". That is, if there are a million civilisations in the Milky Way then at least one of them would have colonised our Solar System by now since the time to colonise the entire Galaxy, with nuclear rockets for example, is far less than the age of the Galaxy. Kuiper referred to Fermi's question as the "big gun in a small arsenal" of arguments in support of $N \sim 1$.

F. Drake of Cornell University occupied the middle ground. The negative results of searches for radio beacons, and radio astronomy in general, imply that N is not very large. But he also argued against $N \sim 1$ and the interstellar colonisation idea on the grounds that colonisation is not cost effective. A better way to improve the quality of life per dollar spent is to remake your own planet and/or solar system. This idea was countered by Kuiper who argued that colonisation would increase the raw material base and by S. von Hoerner of the US National Radio Astronomy Observatory who remarked that luxury makes life nice but "luxury is never cost effective".

P. Morrison of MIT summarised the discussion of N, noting that little new has appeared on the scene since he and Cocconi published the first SETI paper in *Nature* 20 years ago. We have only four 'facts' to go on: Hart's 'fact A' (they are not now here on the Earth), the negative results of radio searches, the presence of life on the Earth, and its absence on Mars. We are still not in quantitative control of the problem, Morrison observed, since calculations of N give 2 σ limits between 10^2 and 10^9. As he did so long ago, Morrison argued for modest experiments with existing radio telescopes to bound the problem observationally.

The second session was devoted to radio searches for extraterrestrial intelligence (SETI). V. Troitsky of the Scientific Radiophysical Institute in Gorky outlined Soviet plans for a fully automated and continuous SETI programme using widely separated antennas on the ground linked with a 10-m dish in space. Troitsky and Shklovsky were two members of a sizeable Soviet delegation at the General Assembly which convened in August in Montreal, Canada. B. Zuckerman (University of Maryland) described recent and ongoing SETI programmes in the USA and Canada and S. Gulkis (Jet Propulsion Laboratory) summarised a SETI programme proposed for inclusion in the NASA FY'82 budget. This 6-year programme, a joint endeavour between NASA's Ames Research Center and JPL, would attempt to optimise the probability of detection using existing radio telescopes in conjunction with new low noise receivers and extremely large digital spectrum analysers.

Jesse Greenstein chaired a discussion on the prospects for detection of simple forms of life elsewhere in the Solar System and the discovery of extrasolar planets. He seemed to respond to Morrison's lament over the lack of progress, noting that SETI as a science was in the awkward position of being "respectable but not yet funded". At least in the case of planetary detection, it was clear that funding would produce definitive answers to a limited set of interesting questions in the near future. G. Soffen from NASA-HQ reviewed the biological ramifications of "life as we know it" and the possibility of recognising "life as we don't know it". T. Owen from the State University of New York pointed

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J. Tarter is at the NASA Ames Research Center and B. Zuckerman is at the University of Maryland.

100 years ago

Change of Colour in Frogs

It is certainly a common opinion in this part of the country that when frogs become of a bright yellow colour fine weather may be expected. The brightness of colour can scarcely be due to the presence of sunlight, for frogs of a bright yellow may frequently be found in cellars, wells, and other dark places. Throughout the past summer and up to the present time, I have noticed that the frogs in this neighbourhood have been of an extraordinarily brilliant yellow tint. Again and again have I heard the country people, working in the hay or corn fields, under the unbroken canopy of cloud, remark — "We must be going to have fine weather now, for look at the colour of the frogs." These forecasts proved the reverse of successful.

At the commencement of "the rains" (say, beginning of June), in the island of Bombay, after the first showers, when a little water lodges in the depressions of the old-quarry tanks, the frogs issue from the crevices of the trap-rock to spawn, when the *males* (some of which are 18 inches in length from tip of toe to end of digit) assume a bright mustard-yellow colour, while the females remain brown as usual; and this change of colour takes place so rapidly, and the frogs are so numerous, that, with the falling of the showers, the bottom of the quarry becomes suddenly yellow.

From *Nature* **20**, Oct 16, 580; 1879.

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out the spectroscopic potential for detecting abundant quantities of O_2 or other disequilibrium atmospheric constituents, indicative of the presence of some form of life.

As for finding extrasolar planets, indirect schemes based on state-of-the-art astrometry, interferometry, radial velocity measurements and the wide field CCD cameras on the Space Telescope were described in turn by G. Gatewood (Allegheny Observatory), D. Currie (University of Maryland), I. McLean (University of Arizona) and W. Baum (Lowell Observatory). Comparing these techniques D. Black of Ames noted their complementarity due to the intrinsically different dependences on stellar mass and planetary size and orbit sampled by the various methods. He concluded that the milli-arcsecond spatial resolution and the 10 m s^{-1} velocity precision needed to detect a Jupiter-Sun system in the local solar neighbourhood are at hand. However direct detection schemes in the visible or infrared and the micro-arcsecond/10 cm s^{-1} precisions required to detect an Earth-Sun system must await the next generation of instrumentation. While this entire field looks extremely hopeful it was suggested by the participants that it may require a 'dialectic unification' to get two different groups of astrometrists to agree on the question of the existence of a planet orbiting around Barnard's star. ☐

The answer lies in the blood

by Mary Lindley

Tiny, dried up bloodstains of indeterminate age may not be the biochemical geneticist's dream, but for those who work in the forensic world they are routine specimens. The continual challenge of obtaining more information from less and less material was in the minds of delegates at a recent international gathering of forensic haemogeneticists*. With the elucidation of the kinetics of the reaction between antigen and antibody, it has become possible to identify blood group antigens immunologially with greatly improved efficiency. P.J. Lincoln (London Hospital Medical College) explained that by careful manipulation of *p*H, ionic strength and the amount of antiserum used, it is possible to identify an antigen on a single bloodstained thread, so that even a minute bloodstain can provide sufficient material to examine for several antigens. Sixteen red cell antigens, including those of the A,B,O and Rhesus systems, can be identified by an elution technique. The thread is incubated with a specific antibody, which is subsequently eluted and examined by a sensitive agglutination test. Antibodies corresponding to the antigens in the bloodstain bind to the dried blood, are recovered by elution and detected in the eluate. The serum markers of the Gm and Km system, determining differences in immunoglobulin constant regions, can be detected by inhibition tests. Antiserum is incubated with the bloodstained thread and if antigen is present, the antibodies are absorbed and the activity of the antiserum inhibited.

Some antigens remain detectable by these methods for only a short time, while others such as ABO can be detected after many months, or even years. The age as well as the amount of material available thus determines the antigens used to establish the origin of a bloodstain. A combination of a few of these antigens, Lincoln said, can distinguish between the bloods of two individuals in nearly every case.

Inhibition tests also form the basis for detecting HLA antigens in bloodstained thread, described by D. Hodge (London Hospital Medical College). This work is very preliminary and so far only the HLA-A antigen has been tried, largely because it is well represented in European and Caucasian populations and because good antisera were available. Best results have been obtained when several antisera have been used in parallel to type a stain. Even assuming success, Lincoln said, HLA typing of bloodstains is unlikely to become routine in casework in the near future but it could provide a valuable specialised proceedure.

More than 20 systems of enzyme polymorphisms can be identified in bloodstains as a result of recent improvements in electrophoresis and the introduction of isoelectric focusing, described by B.J. Culliford (Metropolitan Police Forensic Science Laboratory, London). Using 18 such systems, he and his colleagues know that when they identify a bloodstain according to its profile of enzyme polymorphisms it has come from no more than one in 1,800 people. When a specimen is very meagre, it can be tested sequentially for two or more systems. G.B. Divall (Metropolitan Police Forensic Science Laboratory) said that one piece of bloodstained thread can be used first to identify Gm, Km and enzyme polymorphisms by electrophoresis, and then A,B, and O by an absorption-elution technique.

Early results reported by B. Brinkmann (Institüt für Rechtsmedizin, Universität Hamburg) suggest that measurement of enzyme activities could be a guide to the age of a bloodstain. In test bloodstains the activities of acid phosphatase, phosphoglucomutase, adenylate kinase, adenosine deaminase, and phosphogluconate dehydrogenase declined, rapidly at first and then more slowly, at rates correlated with the enzyme phenotype and the time and temperature of previous storage. Much more has to be learnt about the effects of environmental conditions, however, before any routine application for assessing the age of bloodstains would be possible.

J.L. Thomsen (University Institute of Forensic Medicine, Copenhagen) reported his success in identifying the Y chromosome of leukocytes extracted from bloodstains. He has managed to obtain sufficient leukocyes to treat them with the fluorescent stain that reveals the Y, the only chromosome identifiable in dead cells. In spite of the difficulty of the procedure, Thomsen suggested that it could offer a reliable means of sex determination in blood. His claim to have obtained no false positives was greeted with some scepticism however.

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*The Eighth International Congress of the Society for Forensic Haemogenetics was held in London on 23-27 September, 1979. The congress presidents were Professors B.R. Dodd and C.P. Engelfreit. The proceedings will be published by the Society and further information can be obtained from Dr P.J. Lincoln, Department of Forensic Medicine, London Hospital Medical College, Turner Street, London E1.

Mary Lindley is an Assistant Editor of Nature.

The nucleolus at Weimar

from E. G. Jordan

THE workshop* opened with an outline of our present understanding of nucleolar structure given by M. Bouteille (University of Paris) in which he emphasised the importance of the identification of the discrete pale-staining islands of the nucleolus as the nucleolus-organising regions. These areas which have been given many different names in the past, are clearly distinguishable from the two major parts of nucleoli, the fibrillar and the granular components. P. Hernandez-Verdun (University of Paris) showed that in micronuclei induced with colchicine treatment no organisation of nucleolar material into nucleoli occurred in the absence of such a pale-staining 'fibrillar centre', hereby shown to be functionally related to the nucleolus organisers. C. Mirre (Faculty of Medicine, Marseille) reported that the number of fibrillar centres contributing to the generation of a new nucleolus in meiotic cells was the same as the number of organising chromosomes involved. Many workers agreed that the use of the silver staining procedure identifies these 'fibrillar centres' and hence the nucleolus organisers. However, the specificity of the silver stain is known to depend critically on the conditions used and some workers reported silver staining of structures other than nucleolus

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*The Sixth European Nucleolar Workshop was held in Weimar on 1-6 July, 1979 under the auspices of the European Cell Biology Organisation (ECBO) and was organised by Dr. S. Rosenthal of the Central Institute of Molecular Biology, Academy of Sciences GDR, Berlin.

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organisers, while Y. Daskal (Baylor College of Medicine, Texas) reported that the group of H. Busch had now identified a nucleolar acidic protein with especially high affinity for silver.

The question of the function of the nucleolus-organising regions was raised by G. Goessens (University of Liege, Belgium) who has shown by autoradiography that these regions, or at least those parts which can be identified as pale-staining islands within the nucleolus, are not themselves the sites of RNA transcription. This was further emphasised by W. Franke (German Cancer Research Centre, Heidelberg) who described a specific fibrillar nucleolar skeleton which constitutes a large proportion of the nucleolar material and may be a component of the structures identified as organisers.

The general importance of skeletal structures in understanding the organisation of chromatin and transcriptional processes in the nucleus was emphasised by R. Hancock (Swiss Cancer Institute, Lausanne) who presented evidence to show that the chromatin of the nucleus is arranged in loops which are anchored by regions of repetitive DNA sequences into a peripheral nuclear skeleton consisting of protein distributed in a broad zone around the nuclear envelope. The proteins, which include some prominent polypeptides with molecular weights in the 65,000-75,000 region, are responsible for maintaining a condensed network of chromatin fibrils after the removal of histones, nuclear membranes and pore complexes. The chromatin loops extending from this peripheral skeletal layer ranged from 20-150 kb with an average of about 53 kb, and were displayed using the Kleinschmidt spreading technique. The region of transcription could be identified by the presence of nascent transcripts and was usually found a good distance from the region containing the anchorage sites. The possible relationship of this peripheral skeletal structure with matrix components of the nuclear interior was discussed by I. Zbarsky (Academy of Sciences, Moscow). The possibility that a large part of the nucleolus is constituted by a proteinaceous matrix provides a cautionary note against oversimplified interpretations of nucleolar organisation. While it is clear that in active nucleoli the densely stained fibrillar component surrounding the fibrillar centres of the organiser is the site of transcription, the arrangement of structures involved in maturation and processing stages and their possible association with a nucleolar skeleton are still questions for the future.

Structure of rRNA genes

Intervening sequences (introns) in rRNA genes have been well-characterised over the past few years. At this meeting R. Renkawitz-Pohl (University of Dusseldorf) demonstrated two types of intervening sequences in the rDNA of *Sciara coprophila*, suggesting that the three adjacent groups of ribosomal genes in this organism may differ with respect to these sequences. Heterogeneity between different nucleolar organisers in *Drosophila hydei* was reported by W. Kunz (Dusseldorf). Here the two organisers in the Y chromosome lack a 5.5 kb insert which is found in the rRNA genes of the X chromosome.

The complete 407 bp sequence of the 25S rRNA intervening sequence from *Tetrahymena pigmentosa* was reported by M. A. Wild (Yale University). The intron-exon junctions have been identified and are completely different from those reported for mRNA inserts, which strongly suggests a different splicing enzyme for rRNA. In addition, the position of the splice point is unequivocal as there are no directly repeated sequences at the junctions as there are in mRNAs.

Direct evidence that such a ribosomal RNA gene insert (in this case from the related species *T. thermophila*) is transcribed and processed was provided by W. A. Eckert (University of Heidelberg). The intervening sequence seemed to be removed rapidly as the first step in processing, since the insert was only found in about 20-30% of the largest precursor rRNA fraction. This also suggests that the excising activity resides in the nucleolus.

Homologies between the rDNA of widely different organisms were shown by R. Gourse (Brown University, Providence). By hybridising labelled cloned restriction fragments he found that the rDNA immediately adjacent to the *Tetrahymena* intron was homologous to fragments at similar positions in the rDNA of yeast and *Dictyostelium*. In addition, homologies between the latter two organisms were present at the 3′ end of the region coding for 18S rRNA.

R. Bach (University of Geneva) also reported that several strongly conserved regions had been found in cloned rDNA fragments from three species of *Xenopus*. These conserved regions include the 3′ end of the 40S rRNA precursor and, interestingly, a 30 bp region about 100 bp away from this end. The region immediately preceding the beginning of the precursor was also highly conserved, but, aside from the first few bases, the 5′ end of the precursor itself was not. These findings indicate that the search for 'promoter' sequences may soon be successful.

Interest in the regulation of transcription has led to studies such as those reported by I. Financsek (Institute of Radiobiology, Budapest), who has cloned rDNA fragments from Novikiff hepatoma cells. One clone bound *E. coli* polymerases *in vitro*, and is thought to contain the promoter. Along similar lines, M. Muramatsu (Cancer Institute, Tokyo) has cloned a long mouse rDNA fragment which extends from the middle of the 18S rRNA gene region through the transcribed leader of the pre-rRNA, and probably also contains the promoter.

Such studies are being complemented by functional assays of rDNA in eukaryotic cells, such as its microinjection into amphibian oocyte nuclei.

Pre-mRNA and informosomes

The fate of the primary RNA transcript was discussed in relation to the concepts of pre-message, informosomes and the ubiquitous perichromatin granules. Some equivalence in secondary structure of adjacent RNP fibrils in spread *Drosophila* germ cell chromatin was reported by K. Glatzer (University of Dusseldorf) who calculated a DNP/RNP length ratio of 4.6 showing a similar foreshortening previously described in nucleolar genes. The addition of protein to the early transcripts is thought to result in the formation of granules or particles of RNP. There were two extensive reports on the 'perichromatin granules', PCGs. E. Puvion (Cancer Research Institute, Villejuif) showed how the numbers of these 40 nm granules with their characteristic clear zone or 'halo' could be increased dramatically by reducing the RNA processing activity of cells using cadmium chloride. The PCGs were found all around the blocks of dense chromatin, that is, the site where RNA could also be shown to accumulate by autoradiography at the electron microscopic level. Under these conditions the normal migration of label to the interchromatin spaces was arrested. These granules could also be shown to be connected to chromatin fibrils by slender stalks, using either spread preparations or thin section procedures, which may further indicate that they are modified products of transcription.

Y. Daskal (Baylor College of Medicine, Houston) has isolated one major polypeptide of 40,000 molecular weight and two minor ones (31,000 and 38,000 molecular weight) from PCGs. Interestingly, PCGs also contain a characteristic 4.7S RNA. While these findings are all compatible with the concept that PCGs are a sub-population of mRNP particles more direct evidence of their possible mRNA components is still lacking.

This year's workshop was the first since the death of the founder, Wilhelm Bernard. His unforgettable qualities were recalled by Francoise Hagenau (University of Paris) who reminded us of his deep interest in the humanities and philosophy and his particular admiration of Goethe, who had lived at Weimar. At the workshops he sought always to unite different scientific specialities and different people and nations to make every worthwhile contribution part of an amicable synthesis. □

E. G. Jordan is in the Biology Department, Queen Elizabeth College, London.

articles

A deep-sea hydrothermal site on a strike-slip fault

Peter Lonsdale

University of California, San Diego, Marine Physical Laboratory of the Scripps Institution of Oceanography, La Jolla, California 92093

Submersible exploration of a young scarp in the San Clemente fault zone, at 1,800 m in the California Borderland, discovered tall piles of hydrothermal barite and dense colonies of large benthic animals. Phenomena along this active strike-slip fault zone resemble those at hot springs along the axes of mid-ocean ridges.

DIVE 355 of the US Navy submersible DSV-4 Seacliff, a half-sister of DSRV Alvin[1], landed at 1,805 m on the sandy suprafan of the Navy deep-sea fan[2], 70 km south-west of San Diego, California, in the South San Clemente Basin. The original intent of the dive was the geological examination of large-scale scour marks that have been cut by turbidity currents and mapped by a deep tow instrument[3]. Navigation was by echo-ranging to the Loran C-positioned mother ship, and by piloting on the deep tow chart. After travelling east across the hummocky fan surface for 1 km, the submersible obliquely ascended the 50–75 m scarp of a south-east trending fault (Fig. 1). The scarp has a clearly defined foot lapped by fan turbidites, and an abrupt crest at the top of a 60° slope. Although acoustic profiles (Fig. 2) indicate that the scarp truncates back-tilted beds of layered sediment, no semi-lithified outcrops were visible; the scarp surface is covered by silty mud, with signs of local mass-movement of unconsolidated sediment down its steepest segments. Both on the fan surface and on this part of the scarp the type and abundance of animal life seemed normal for basins in the California Borderland, with mobile holothurians, ophiuroids and quill worms the most obvious megafauna.

After climbing to the crest of the narrow fault ridge, Seacliff turned south and recrossed the scarp. At a position of 32°13.6′ N, 117°43.5′ W, about 500 m south-west of the first crossing, the foot of the scarp is marked by an uneven line of cones, columns and irregular piles of mineral deposits (Fig. 3*a*, *b*). They generally rise 2–3 m above the sediment surface, but one large mass has an estimated height of 10 m. Some of the deposits are white, and have a very uneven surface with many fingers and protuberances, but many of the larger masses have a smoother dark brown surface and a patchy dusting of sediment. The dark crust was easily broken by the submersible's manipulators, to reveal friable white interiors. Higher up the scarp, extending about half-way to the crest, are smaller white deposits, commonly seen as irregular fingers less than 1 m long projecting through the mud (Fig. 3*c*, *d*). Around some of these deposits the buff-coloured sediment bears thin patches of dark-brown soot-like material, the water within a few centimetres of white surfaces has a milky appearance, and there are dense colonies of large tube worms and other animals.

The mineral masses proved to be made of barite crystals. Although we lack direct evidence of anomalous bottom water, having had no water sensors or samplers on Seacliff, the phenomena at this site are interpreted as effects of mineralised thermal springs discharging along the fault line.

After almost an hour of photography (with hand-held 35 mm cameras through the observer's port) and sampling, the dive was curtailed because of mechanical difficulties. How far the baritic sinter and tube-worm clusters extend along the fault line was not determined, but it is at least 100 m.

Regional setting

The scarp at Navy Fan has been created by a component of dip-slip motion along the predominantly strike-slip San Clemente fault zone. Local vertical displacements are common along California strike-slip faults, with the uplift alternating from one side to the other along the fault trace; 100 km north-west of our study area tilted San Clemente Island has been formed by relative uplift of the opposite side of the fault. The fault ridge we examined preceded and steered the post-22,000 yr BP growth of Navy Fan[2], but continued motion on the fault line may be indicated by low scarps and lineations mapped by side-scan sonar on the fan surface north-west of the ridge[3]. We lack seismic profiles across the dive site, but the basement comes within 100 m of the sea floor at a crossing of the fault 5 km to the south-east[4]. The nearest sampled basement outcrop is at Navy Bank, where andesites have been dredged[5]; the basaltic andesites of San Clemente Island have been dated at 15–16 × 10^6 yr BP[6].

The San Clemente fault zone is but one of the several simultaneously active shear zones that make up southern California's broad strike-slip 'boundary' between the Pacific and American plates. It was once thought to connect with the Agua Blanca fault zone[3], but recent seismic reflection surveys[7] indicate that the principal fault trace is well offshore at Punta Banda, and continues south-south-west along the margin of Baja California (Fig. 1). The Miocene lavas of San Clemente Island are manifestations of widespread basaltic volcanism that was associated with plate-boundary tectonism in mid-Miocene to Pliocene times. The tholeiites, alkali basalts and basaltic andesites throughout this province are notable for their unusually high contents of barium[8,9]: for example, tholeiitic and transitional basalts from the Jacumba volcanics have up to 0.3% barium, more than 200 times the average for mid-ocean ridge tholeiites[8].

Quaternary igneous activity has probably been restricted to the Salton Trough, where incipient seafloor spreading may be taking place[10], but the geothermal gradient remains above average in much of the region[11]. There are many subaerial thermal springs with temperatures of 30–60 °C, concentrated

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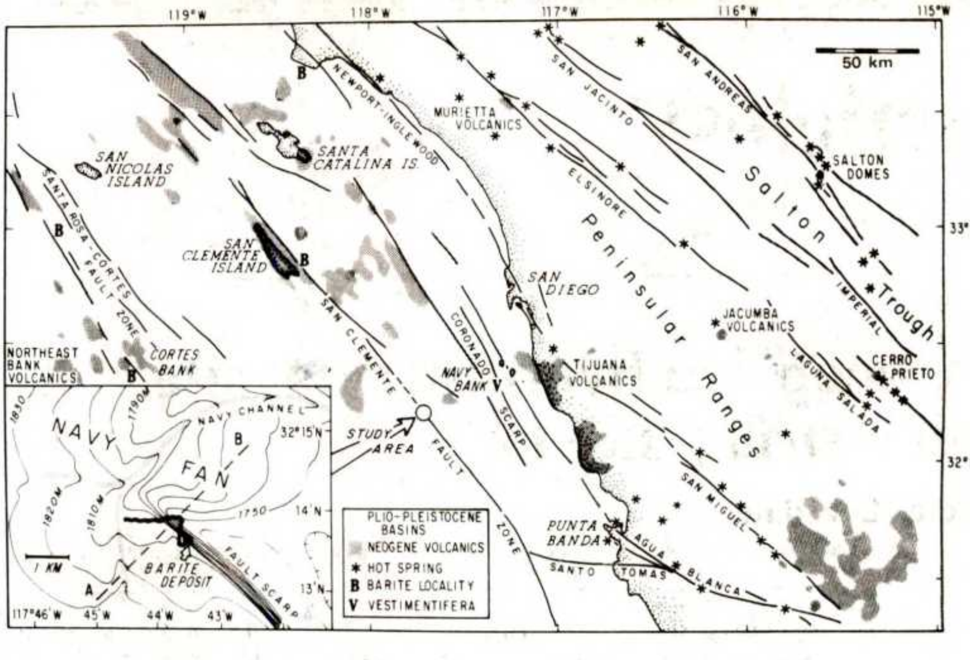

Fig. 1 Location map, showing principal Quaternary faults (after refs 5, 7, 33 and 34), offshore barite occurrences and the Vestimentifera type-locality[18]. Inset, with bathymetry from a deep tow survey[3] shows submersible track (solid line) and the position (A–B) of the 3.5-kHz profile of Fig. 2.

along the strike-slip shear zones (Fig. 1) where shattered bedrock and faulted alluvial cover are more permeable and frictional shear heating might be a significant local heat source[12]. Many of these springs deposit travertine. Where the Agua Blanca fault zone crosses the coast at Punta Banda, chemically-modified seawater flows from littoral thermal springs at temperatures of over 100 °C, and precipitates pyrite and gypsum[13]. Along the east coast of the Baja California peninsula some littoral springs deposit barium sulphate as well as calcium sulphate, most notably at Puertecitos (30°20′ N, 114°38′ W) where barite and pyrite forms extensive deposits around a thermal discharge from faulted Miocene and Pliocene volcanic rocks[14].

There have been no reports of active thermal springs in the Borderland, comparable to those in the same structural setting on land, but, of course, the problems of locating them in the deep sea are much greater. For many years it has been known that barite nodules occur within the mud at some of the Borderland's fault zones, and Revelle and Emery[15] interpreted samples from the San Clemente Island fault scarp as hydrothermal precipitates, formed when hot mineralised chloride solutions encountered sulphate-rich interstitial brines. Other suggestions for their genesis have included precipitation in evaporite basins[16,17]. More recently, porous masses of barite crystals, similar to those recovered by Seacliff Dive 355, have been dredged beside Cortes Bank in the Santa Rosa–Cortes fault zone[5] and on the Patton Escarpment (J. W. Hawkins, personal communication). We interpret these fragile crystalline masses as primary baritic sinters precipitated around thermal springs (hydrothermal vents) on the seabed. The nodules probably contain recemented barite reworked from older deposits of similar origin; the locality dredged by Revelle and Emery was reexamined and resampled in July 1979 on Alvin Dive 944, but no signs of recent hydrothermal activity were seen.

Sample description

Seacliff recovered 5 kg of barite from two separate piles. They are porous, open meshworks of small (10–100 μm) tabular crystals, and have a low saturated bulk density (1.6–1.8 g cm^{-3}) despite barite's high specific gravity (4.5). The barite crystals have a pearly lustre, and are grouped in a variety of dendritic and fan-shaped arrangements (Fig. 4). The crystal masses are contaminated by mud-filled burrows where worms have penetrated the friable material, but are otherwise very pure. No other mineral phases could be identified by X-ray diffraction. Chemical scanning for elements other than barium and sulphur, using an X-ray energy dispersive attachment to a scanning electron microscope, discovered significant amounts (>1% in some patches) of only iron and aluminum. Energy-dispersive X-ray spectrometry revealed only 0.2% strontium. Iron is probably present as fine-grained hydroxides, as some parts of the crystal masses have a light orange stain. One sample has a dark chocolate crust 1 mm thick. It is made of manganese oxide, contaminated with detrital silicate minerals but with a low iron

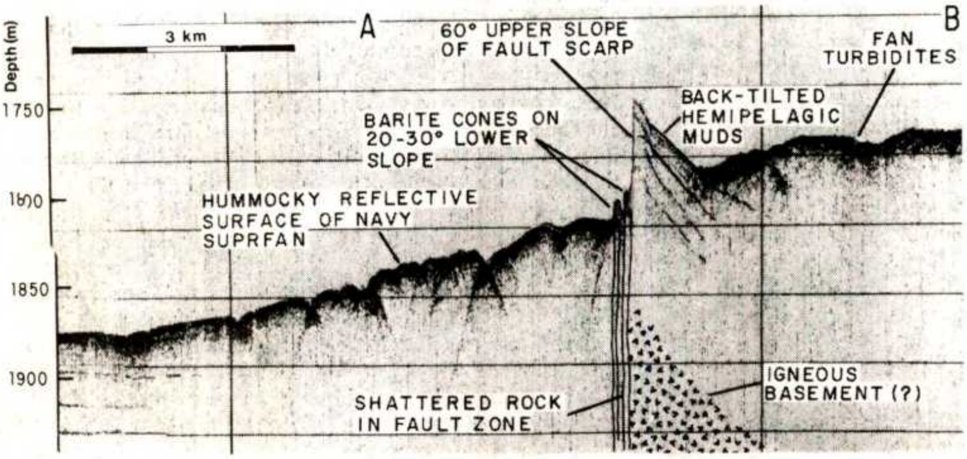
Depth (m)
1750
1800
1850
1900
3 km
A
B
60° UPPER SLOPE OF FAULT SCARP
FAN TURBIDITES
BACK-TILTED HEMIPELAGIC MUDS
BARITE CONES ON 20-30° LOWER SLOPE
HUMMOCKY REFLECTIVE SURFACE OF NAVY SUPRFAN
SHATTERED ROCK IN FAULT ZONE
IGNEOUS BASEMENT (?)

Fig. 2 3.5-kHz acoustic profile across the San Clemente fault, on line A–B of Fig. 1 (inset). At the scarp, where the steep slopes returned no echo on the original record, features have been sketched on from visual observations; structures sketched beneath the fault scarp are inferred from a nearby reflection profile[4].

content. Despite its thinness, this crust helps hold the loose mass of barite crystals together, and must increase the resistance and persistence of the tall barite cones.

Benthic fauna

The most prominent animal life on the barite deposits and the intervening sediment slopes are dense tangles of tube worms. Many of the colonies near the large, brown-crusted deposits at the foot of the scarp may be dead: their crooked tubes are dusted with sediment and thickly festooned with epizoa, and no bodies were seen protruding from the tubes (though they could have been retracted inside). Near the fresher white deposits higher on the slope (for example, Fig. 3*b*, *c*) the tubes (many of them more than 1 m long and averaging 1 cm in diameter) tend to be straighter and less encumbered with epizoa, and several had dark red 'plumes' protruding from their tips. The animals were sampled, but their soft parts were not preserved; from their tubes and growth habit they are identified as the vestimentiferan *Lamellibrachia barhami*. Descriptions of this gutless annelid (?)[18,19] rely on specimens observed and collected by submersible at 1,125 m on the foot of Coronado Scarp, a fault scarp on the east side of San Diego Trough about 40 km from the Navy Fan site. Its only known close relatives are the Vestimentifera clustered around some thermal springs on mid-ocean ridges[20].

Unidentified bluish-grey organisms form apparently gelatinous masses draped over some barite surfaces and many of the chitinous tubes of the Vestimentiferae we photographed. Some of the sampled tubes had Bryozoa colonies. Large stalkless crinoids were attached to the tops of several barite cones (see Fig. 3*b*). Carpeting the sediment surface near some irregular white projections of barite were clusters of gastropods 4–5 cm long (Fig. 3*c*) and the valves of bivalve molluscs up to 10 cm long (Fig. 3*d*). Other animals seen around the barite deposits, but with an abundance not noticeably greater than elsewhere on the fan, include ophiuroids, holothurians, several types of fish and a large blue octopod (nestling in a barite concretion).

Interpretation and implications

The sinter-like deposits of barite on the floor of a Borderland basin are precipitates from mineralised springs along an offshore part of California's strike-slip fault system. Their evident capacity for carrying large amounts of barium in solution indicates that the springs were discharges of reduced hydrothermal fluid. Recent precipitation is attested by the fragility of the deposits and the absence on many surfaces of any coating of manganese oxide or sediment. The best signs of continuing active hydrothermal discharge, in the absence of direct temperature measurements, are the abundance of animals whose feeding habits attract them to thermal springs, and the milky appearance of bottom water in contact with some barite piles, reminiscent of the hydrothermal plumes at mid-ocean spreading centres[20]. By extrapolation, other places in the Borderland with nodular or massive barite are inferred to be sites of past or present thermal springs, as is the type-locality for *Lamellibrachia barhami* on the Coronado Scarp.

Several features of the continental margin site explored by Seacliff are comparable to the more spectacular deposits of oxides, silicates and sulphides and the dense clusters of large organisms found around thermal springs at axial fissures and strike-slip faults on mid-ocean ridges[20-24]. Seawater-based hydrothermal fluids that have reacted with young oceanic tholeiites at the Galapagos spreading centre[20] have the high barium enrichments predicted from laboratory studies[25], but no local accumulations of barite have been described. Barite precipitation has been reported from an actively spreading marginal basin[26], while dispersed barite crystals of probable hydrothermal origin are found over a large area of the East Pacific Rise[27], and in the Red Sea's metalliferous muds[28], where calcium sulphate is much more abundant.

The environment of thermal springs along the San Clemente fault one is different from those at accreting plate boundaries in that the geothermal gradient is much lower and the deep convective circulation must be much weaker. However, an active hydrothermal system has already been inferred for the San Clemente fault zone[29] from the pattern of conductive heat flow, which includes measured fluxes as high as 3.2 μcal cm^{-2}. The rocks and sediment available for reacting with hot, reduced seawater are also different from the sediment-free oceanic tholeiite of most mid-ocean ridges. Dominance of barite precipitation is attributable to the high concentration of barium in the local Neogene volcanic rocks.

In the convective circulation proposed for the San Clemente fault zone, seawater that penetrates shattered bedrock would have its sulphate reduced by reaction with volcanic rock, from which barium would be stripped by the chloride solution. A

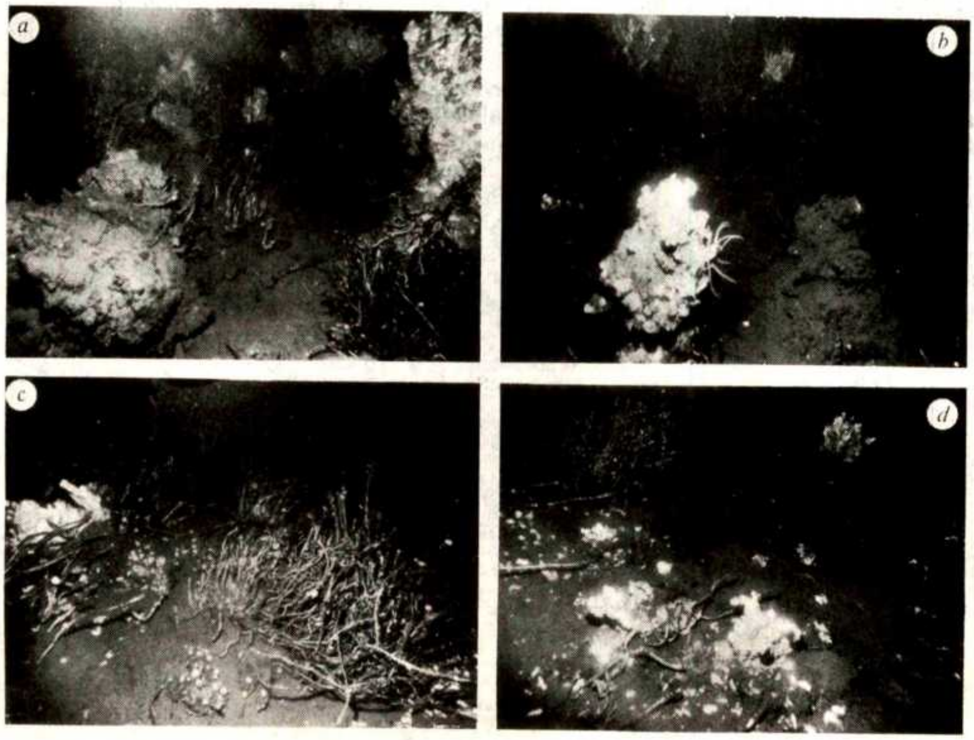

Fig. 3 Reproductions of colour photographs of the barite deposit, taken with a hand-held 35 mm camera through Seacliff's observation port. Field of view is about 3 m wide in mid-field. *a*, Irregular barite mounds 1–2 m high at the foot of the fault scarp, with tangled tubes of probably dead Vestimentiferae. *b*, Contrasting appearance of two barite mounds, one white and irregular (with a large stalkless crinoid), and one darker, smoother and veneered with sediment. *c*, the 30° lower slope of the fault scarp, with protruding 50 cm high 'fingers' of white barite, abundant tube worms (some definitely alive, with protruding red bodies), and clusters of white gastropods (on the sediment, barite, and some Vestimentifera tubes). *d*, Irregular clumps of barite, surrounded by milky water (poorly seen in this black and white reproduction), clusters of empty clam shells, and living Vestimentiferae. Irregular patches of dark, sooty sediment visible on the slope.

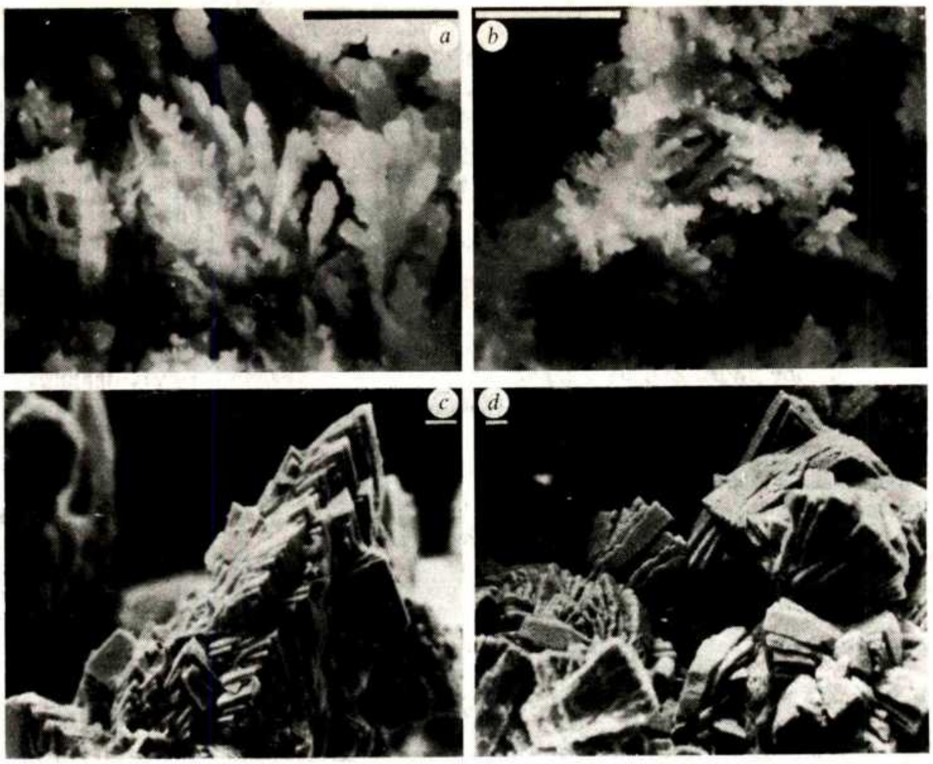

Fig. 4 Internal structure of barite crystal masses, as seen by a reflected light microscope (*a*, *b*; scale bar, 1 mm) and scanning electron microscope (*c*, *d*; scale bar 10 μm); platy crystals form spires and frondose structures projecting into internal cavities.

conduit for discharge through the relatively impermeable sediments is provided by the fault, which probably resembles strike-slip faults on land in being a belt of shearing 100–200 m wide, overlying a similar width of crushed bedrock. Precipitation of barite, which is undersaturated in normal seawater, would occur where barium-rich hydrothermal fluids mix with sulphate-rich bottom water. The large number of separate mounds of barite indicates a more diffuse discharge than occurs at ridge–crest hot springs, where there is no sediment cover to fractured bedrock. Because the crystal masses are so porous the precipitated mounds do not impede the discharge and seal the system. Those mounds with fresh white exteriors are believed to be slowly leaking barium-rich water, and growing barite crystals on their surfaces; their dynamics may be similar to the slow discharge through the porous deposits of manganese, iron and silica at the Galapagos Mounds Hydrothermal Field[20,30]. Manganese oxide precipitation on the mounds apparently occurs after outflow of barite-precipitating fluid has ceased, and does effectively decrease surface permeability and seal the discharge.

Iron and aluminum in the barite deposits has probably also been leached from underlying igneous rocks[15], which are also the most likely source of encrusting manganese. The dark sooty sediment nearby may be a loose precipitate of manganese oxide. Elsewhere, baritic sinters are often associated with sulphide ores of more valuable metals. In northeastern Honshu, barite occurs as onshore hot spring deposits, offshore nodules, and as the capping of Kuroko ore bodies–stratiform deposits of copper–lead–zinc sulphides formed at bathyal depths by Miocene hydrothermal precipitation in offshore basins overlying rhyolite and andesite[31,32]. Just as hydrothermal vents on the East Pacific Rise may now be producing mineral deposits similar to the Troodos-type massive sulphides[22], so thermal springs in the basins of the California Borderland may be modern ore-depositing counterparts of the Kuroko hot springs. The subaerial hot springs of Baja California have previously been suggested as the loci for active accumulation, at depth, of sulphide ores[33]; the prospective value of seawater-based hydrothermal systems like those in the Borderland is greater because of their enhanced ability to transport metals in chloride complexes. Establishing the abundance of mineralised thermal springs in the Borderland will require high-resolution observations along the fault zones there; in the submarine environment they are unlikely to be as far apart as the 10–50 km that separates fault-zone thermal springs on the adjacent semi-arid land (Fig. 1).

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Modification of the oxygen-isotope record in deep-sea cores by Pleistocene dissolution cycles

Jonathan Erez*
Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543

The glacial–interglacial amplitude (GIA) of oxygen-isotope curves in sediment cores from the Atlantic Ocean is much higher than the amplitude in the Pacific. This difference is explained by nonsynchronous $CaCO_3$ dissolution cycles during the Upper Pleistocene that amplify the GIA in the Atlantic and reduce the GIA in the Pacific. Efforts to assess palaeotemperatures in the Pleistocene using the stable isotope technique must consider the effects of dissolution.

MUCH of our knowledge on the Pleistocene epoch and its glaciations comes from oxygen isotope studies of foraminiferal skeletons from deep-sea cores. In addition to being a powerful stratigraphic tool[1–4], the oxygen isotope record provides information on the timing of glacials, sea-level fluctuations, palaeotemperatures, palaeosalinities and ice accumulation and melting during glacials and interglacials[1–9]. Deep-sea cores also display cyclic variation in $CaCO_3$ content. These $CaCO_3$ cycles have a periodicity similar to that of the Upper Pleistocene glacial–interglacial fluctuations and are probably caused by $CaCO_3$ dissolution[10–13]. The purpose of this article is to show how $CaCO_3$ dissolution cycles have modified the oxygen isotope record in deep-sea cores.

$CaCO_3$ dissolution cycles during the Pleistocene

The mechanism that causes $CaCO_3$ cycles in deep-sea cores is the subject of much debate and one reason for this is the lack of synchroneity between the Atlantic and the Pacific with respect to the timing of these cycles. In the Atlantic $CaCO_3$ content is high during interglacials and low during glacials, while in the Pacific the opposite is observed[10–16]. Variation in the $CaCO_3$ content of oceanic sediments can be caused by changes in $CaCO_3$ productivity, $CaCO_3$ dissolution and dilution by non-carbonate material. All these mechanisms and some of their combinations have been suggested to explain the $CaCO_3$ cycles and to account for the difference in timing between the Atlantic and the Pacific[10–16].

Broecker[14] and Berger[11] have convincingly shown that in the Pacific $CaCO_3$ cycles are caused mainly by dissolution. Their conclusions are based on $CaCO_3$ mass balance and on micropalaeontological criteria. Luz and Shackleton[12] provided additional evidence for $CaCO_3$ cycles in the Pacific being caused by dissolution, and also showed that these cycles lag a few thousand years behind the onset of glacials and interglacials. However, several authors suggested that $CaCO_3$ cycles in the Atlantic are not caused by dissolution, but rather by changes in $CaCO_3$ productivity or dilution with non-carbonate material[12,14,17,18]. More recent work in the Atlantic, especially by CLIMAP[19], has shown clearly that $CaCO_3$ cycles in the Atlantic are caused mainly by dissolution. The evidence for this is summarised briefly below.

Gardner[13] showed that in the eastern equatorial Atlantic, cores from relatively shallow water (~3,000 m) have high $CaCO_3$ content (~80%) and that glacial–interglacial carbonate variations are small. However, with increasing water depth average $CaCO_3$ content decreases and glacial–interglacial amplitudes increase (Figs 3 and 4 in ref. 13). This systematic variation with depth cannot be attributed to changes in productivity because these cores are located close to each other. Carbonate dissolution is strongly controlled by depth (mostly because of pressure increase) and thus can better explain this variation. Gardner[13] has also shown by material balance considerations that dilution alone cannot be responsible for the variation in $CaCO_3$ observed in this region. Following Gardner's calculation[13], it has been shown that at least 60% of the $CaCO_3$ deposited during glacials in the eastern equatorial Atlantic must have dissolved[20]. In addition, Gardner[13] found that benthonic/planktonic foraminifera ratio and shell fragmentation indicate increased dissolution during the low carbonate glacial stages. Bé *et al.*[21] showed a similar pattern in the western equatorial Atlantic. Glacial stages have low carbonate content, decreased coarse fraction, excessive fragmentation of planktonic foraminifera, absence of pteropods and increased benthonic/planktonic foraminifera ratio. Johnson *et al.*[16], Prell and Hays[22] and Thunell[23] found similar dissolution cycles in the southwestern Atlantic, Columbia Basin and the Gulf of Mexico, respectively. Therefore, although productivity changes and

Table 1 Comparison between the oxygen isotope composition of planktonic foraminifera from the uppermost sediment of two box cores in the North Atlantic Ocean, collected at 4,950 m and 4,500 m

Species	OC28-396 $\delta^{18}O$ (4,950 m)	OC28-395 $\delta^{18}O$ (4,500 m)	$\Delta\delta^{18}O$ (4,950–4,500 m)
G. ruber	−0.43	−0.71	+0.28
G. truncatulinoides	+0.98	+0.55	+0.43
G. inflata	+1.01	+0.74	+0.27
G. hirsuta	+1.23	+0.89	+0.34
G. conglobatus	+0.13	−0.20	+0.33
G. sacculifer	−0.55	−0.78	+0.23
G. siphonifera	+0.15	−0.06	+0.21
O. universa	−0.15	−0.20	+0.05
P. obliquiloculata	−0.12	+0.06	−0.18

Note that for all species except *P. obliquiloculata* $\delta^{18}O$ is higher in the deeper box core than in the shallow one. The difference in the isotopic composition is attributed to preferential dissolution of foraminifera that are isotopically lighter.

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* Present address: Hebrew University of Jersusalem, The H. Steinitz Marine Biology Laboratory, PO Box 469, Eilat, Israel.

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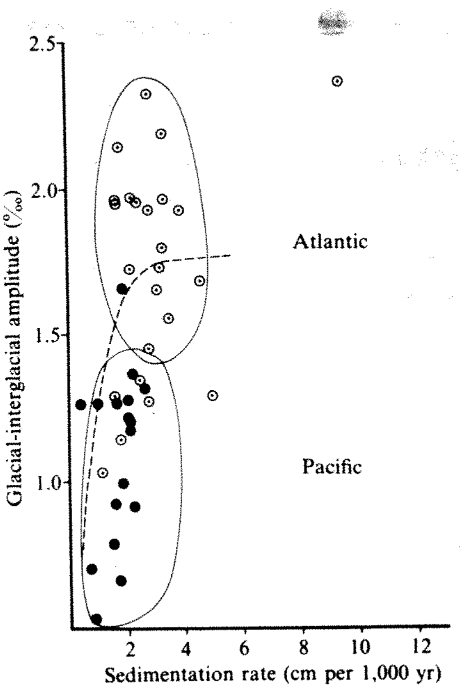

Fig. 1 The glacial–interglacial amplitude (GIA) of oxygen isotope curves in tests of planktonic foraminifera from sediment cores in the Atlantic (⊙) and Pacific (●) Oceans plotted against sedimentation rates in these cores. The isotopic composition is expressed in delta notation,

$$\delta^{18}O = \frac{(^{18}O/^{16}O)\ \text{sample}}{(^{18}O/^{16}O)\ \text{standard}} - 1 \times 1{,}000$$

Sources for the data and calculation of the amplitudes are discussed in Table 2. Note that the amplitudes in the Atlantic are significantly higher than amplitudes in the Pacific. The dashed line is the predicted GIA of a model suggested by Peng *et al.*[57] which accounts for bioturbation at different sedimentation rates. Most Atlantic amplitudes are higher, and Pacific amplitudes lower than the model predicts. The difference in the GIA between the two oceans is attributed to the effects of $CaCO_3$ dissolution cycles on the isotopic record.

dilution with non-carbonate material influenced carbonate content in certain areas, it seems that $CaCO_3$ cycles in both oceans are caused mainly by dissolution; they have similar frequencies as the glacials and interglacials; and the Atlantic and the Pacific are out of phase with respect to the timing of dissolution.

Today's oceans seem to be in agreement with the scheme suggested above. In the present interglacial the Atlantic is in a preservation mode, the CCD is roughly at 5–6 km and the saturation level ($\Omega = 1$) is at 4 km (ref. 24). The Pacific, on the other hand, is in a dissolution mode, its CCD is at 3–4 km and the saturation level ($\Omega = 1$) is at 1–2 km (ref. 24).

Effects of dissolution on the isotopic composition of planktonic foraminifera

Several authors have reported that Recent planktonic foraminifera recovered from the deep-ocean floor are isotopically heavier than their counterparts, recovered from shallower depths[25-27]. It was suggested that this enrichment in ^{18}O is caused by selective dissolution[25-28]. I have compared the isotopic composition of planktonic foraminifera recovered from the uppermost sediment of two box-cores from the north Atlantic (core OC28, 396 from 34°18′ N, 57°12′ W, in 4950 m water depth and core OC28, 395 from 33°43′ N, 57°27′ W in 4,500 m water depth). The results, shown in Table 1, are in good agreement with earlier studies mentioned above[25-27]. All species from the deeper box core with the exception of *Pulleniatina obliquiloculata* showed small but significant increase in $\delta^{18}O$ compared with the shallower box core. Visual observation of the foraminifera in these box cores indeed indicates that the specimens from 4,500 m are better preserved than those from 4,950 m, suggesting that dissolution is the prime cause for the difference in the isotopic composition between these two samples[20].

Planktonic foraminiferal tests collected in plankton tows are thin and fragile[28]. Their oxygen isotope composition is light (that is enriched in ^{16}O) and often in slight negative deviation from equilibrium[29-31], possibly caused by incorporation of isotopically light metabolic CO_2 which is enhanced by their symbiotic algae[20,32]. As foraminifera sink through the water column, part of the population continues to calcify, more chambers are added and the tests become thicker[20,33-37]. The isotopic composition of this part of the population is heavier because its skeleton is deposited in colder water and the activity of symbiotic algae ceases below the photic zone[20,30,38]. Dissolution of the overall population tends to first remove the light-weight fragile individuals which are isotopically lighter[28]. Consequently, the surviving population is isotopically heavier. This scheme which has recently been confirmed by comparison of planktonic foraminifera from plankton tows, sediment traps and sediments[20], explains how dissolution makes the isotopic composition of a species population heavier. It is now possible to examine the effect of $CaCO_3$ dissolution cycles on the isotopic record of the Pleistocene.

Effects of dissolution cycles on the isotopic record of the Pleistocene

When considering this problem, absolute values of $\delta^{18}O$ are of little interest because large variability exists between and within species due to their depth and geographic distribution. It should also be remembered that intercalibration between laboratories is not complete, especially because different treatments are used to remove the organic contaminants before isotopic analysis. For these reasons, I will consider here only the effects of dissolution on the isotopic glacial interglacial amplitude (GIA) which is comparable between species and between laboratories.

Most authors agree that the major factor controlling the GIA is ice volume in the Northern Hemisphere and its isotopic composition[1-3,39-41]. Temperature, assumed to be the main factor by Emiliani[5,6], seems to be of secondary importance (see more recent reviews by Hecht[42], Van Donk[43], and Shackleton[4]). Remarkable similarity exists in the shape and frequency content of $\delta^{18}O$ curves in the Atlantic and the Pacific Oceans. However, there is a large difference in the GIA between the two oceans[1,5,6,41]. Figure 1 and Table 2 summarise most of the pertinent data on the GIA exhibited by planktonic foraminifera. Most Atlantic cores show an amplitude of ~1.9‰, whereas most Pacific cores show an amplitude of ~1.1‰. Emiliani[5,6,44], Imbrie *et al.*[40], Shackleton and Opdyke[2,3], and Berger and Gardner[41], who tried to explain this difference, assumed that the Pacific responded only to the ice volume effect whereas in the Atlantic additional effects of temperature and evaporation/precipitation are superimposed on the ice-volume effect.

I suggest that most of the difference observed in the GIA between the Atlantic and the Pacific is caused by $CaCO_3$ dissolution cycles. Dissolution effects on the isotopic composition of a species population are quite small, perhaps in the range 0.2–0.4‰ (Table 1 and refs 25, 27). However, the timing of dissolution–preservation cycles within and between the two oceans can amplify the effects of dissolution on the GIA. Foraminifera in a well preserved core (even above the foraminiferal lysocline) are already partially dissolved[45], so the isotopic composition of such foraminifera is somewhat heavier than the original composition. Additional dissolution will make the isotopic composition even heavier, while better preservation will make the isotopic composition lighter and closer to the original.

In the Atlantic relatively heavy isotopic composition during glacials will become even heavier due to increased dissolution, and light isotopic composition during interglacials will become lighter because of preservation. Thus the total effect is an

Table 2 Glacial–interglacial amplitude (GIA) of oxygen isotope curves of planktonic foraminifera tests in deep-sea cores from the Atlantic and the Pacific Oceans

Core no.	Depth (m)	Location*	Species	$\delta^{18}O$(‰) Glacial	$\delta^{18}O$(‰) Interglacial	GIA (‰)	Sedimentation rate (cm per 1,000 yr)
Atlantic							
V28-14	1,855	NA	*G. pachyderma*	+4.64	+2.11	2.35	9.4
RC11-86	2,829	SA	*G. sacculifer*	+0.79	−0.55	1.34	2.5
RC12-294	3,308	SA	*G. bulloides*	+2.88	+0.96	1.92	2.8
V19-240	3,103	SA	*G. inflata*	+2.37	+0.72	1.65	3.1
V19-248	3,321	SA	*G. ruber*	+0.90	−0.13	1.03	1.2
V19-282	4,356	EA	*G. dutertrei*	+1.60	+0.31	1.29	5.0
V22-38	3,797	EA	*G. sacculifer*	+0.19	−0.95	1.14	1.8
V22-174	2,630	EA	*G. ruber*	+0.32	−1.23	1.55	3.3
V22-174	2,630	EA	*G. sacculifer*	+0.81	−0.92	1.73	3.3
A172-6	4,160	CR	*G. sacculifer*	+0.09	−1.87	1.96	3.3
A179-4	2,965	CR	*G. ruber*	−0.23	−2.04	1.81	1.8
A179-4	2,965	CR	*G. sacculifer*	+0.16	−1.80	1.96	1.8
A179-4	2,965	CR	*G. dubia*	+0.86	−1.04	1.90	1.8
A180-73	3,749	EA	*G. sacculifer*	+0.51	−1.44	1.95	1.7
234A	3,577	EA	*G. sacculifer*	+0.08	−1.76	1.84	1.2
234	3,577	EA	*G. sacculifer*	+0.15	−1.80	1.95	2.4
235A	4,560	EA	*G. sacculifer*	+0.24	−1.90	2.14	1.8
246	3,210	EA	*G. sacculifer*	+0.04	−1.64	1.68	4.6
280A	4,256	NA	*G. inflata*	+1.92	+0.63	1.29	1.6
P6304-8	3,927	CR	*G. sacculifer*	+0.63	−1.29	1.92	3.9
P6304-9	4,126	CR	*G. sacculifer*	+0.44	−1.35	1.79	3.3
A240-M1	4,180	CR	*G. sacculifer?*	+0.01	−1.71	1.72	2.2
A245 BR-C	2,968	CR	*G. ruber*	−0.30	−1.57	1.27	2.8
V12-122	2,800	CR	*G. sacculifer*	−0.01	−2.19	2.18	3.0
V12-122	2,800	CR	*G. sacculifer*	−0.15	−2.47	2.32	3.0
Pacific							
BNFC43 PG3	2,874	EP	*G. sacculifer*	−0.44	−1.80	1.36	1.1
RC8-94	3,074	SP	*G. sacculifer*	+0.81	+0.05	0.76	1.8
RC10-114	2,791	EP	*G. sacculifer*	−1.04	−1.66	0.62	0.9
RC11-210	4,420	EP	*G. sacculifer*	+0.16	−1.30	1.46	2.2
RC11-210	4,420	EP	*P. obliquiloculata*	+0.45	−0.92	1.37	2.2
RC11-230	3,259	EP	*G. sacculifer*	−0.06	−1.08	1.02	1.7
V21-33	3,726	EP	*G. sacculifer?*	+1.48	+0.18	1.30	2.2
V21-59	2,992	NP	*G. sacculifer*	−0.39	−1.19	0.80	0.8
V21-146	3,968	NP	*G. inflata*	+2.32	+1.01	1.31	2.1
V24-109	2,367	EP	*G. sacculifer*	−0.67	−2.08	1.41	2.7
V28-203	3,243	EP	*G. sacculifer*	−0.45	−1.72	1.27	2.2
V28-235	1,746	EP	*G. sacculifer*	−0.71	−1.80	1.09	1.9
V28-238	3,120	EP	*G. sacculifer*	−0.96	−1.97	1.01	2.3
V28-239	3,490	EP	*G. sacculifer*	−0.84	−1.72	0.88	1.6
WAH-8F2	4,308	EP	*G. obliquiloculata*	+0.73	−0.63	1.36	1.7
Y69-106P	2,870	EP	*G. sacculifer*	−0.20	−1.95	1.75	1.9
Y71-7-45P	3,096	EP	*G. sacculifer*	+0.24	−1.12	1.36	0.5

Data were selected from refs 2–6 and 40. Cores that did not have adequate sampling density or did not show the regular glacial–interglacial pattern were omitted. The amplitude was calculated by the difference between the highest and lowest $\delta^{18}O$ value in the uppermost section of each core, which represents the last glacial and the present interglacial. Sedimentation rate was calculated for the same core section assuming an age of 18,000 ys ago to the peak of the last glacial. Note that the GIA in the Atlantic is almost doubled compared to the Pacific.

* EA, Equatorial Atlantic; EP, Equatorial Pacific; NA, North Atlantic; NP, North Pacific; SA, South Atlantic; SP, South Pacific; CR, Caribbean.

increase in GIA (Fig. 2). In the Pacific the light isotopic composition during interglacials will become slightly heavier due to dissolution while the heavy isotopic composition during glacials will become lighter due to preservation. This will reduce the GIA in the Pacific (Fig. 2). If we assume that dissolution or preservation shifts the isotopic composition only by 0.2‰, the GIA in the Pacific may be reduced by 0.4‰, and amplified in the Atlantic by 0.4‰, thus the difference in the GIA between the two oceans may reach 0.8‰ which is quite close to the observed values (Table 2, Fig. 1).

The following studies by other investigators support my interpretation. Berger and Gardner[41] have suggested that dissolution caused the extremely low GIA in the Pacific (0.2–0.8‰) reported by Emiliani[5]. Shackleton and Opdyke[3] have also shown that within the Pacific, core V28-238 from 3,120 m recorded a larger GIA than core V28-239 from 3,450 m. The average difference in GIA between the two cores was 0.29‰. Shackleton and Opdyke[2] showed that the GIA recorded by benthonic foraminifera in core V28-238 is higher by 0.2‰ than that recorded by *Globigerinoides sacculifer*. They suggested that *G. sacculifer* migrated downwards during the interglacial to account for this observation. While this is not impossible, it is easier to explain this difference by dissolution that reduced the GIA recorded by *G. sacculifer*, but did not change the GIA recorded by benthonic foraminifera which are more dissolution resistant.

Berger and Killingley[27] have recently compared the isotopic record of the past 19,000 yr in two western equatorial Pacific box cores from 1,597 m and 3,732 m. They showed that the isotopic composition of *P. obliquiloculata* (a dissolution-resistant species) was unaffected by dissolution, while the isotopic composition of *G. sacculifer* (which is dissolution susceptible) was significantly heavier in the deeper box core due to dissolution during the past 10,000 yr. Thus the GIA recorded by *G. sacculifer* in the deeper core is lower by 0.3‰ than the GIA recorded by *P. obliquiloculata*.

In the Atlantic, it is expected that dissolution resistant species will show a smaller GIA than dissolution susceptible species. Indeed, in the Caribbean core A-179-4 (from 2,965 m), Emiliani[5] showed that while the dissolution susceptible species *G. ruber* and *G. sacculifer* had high GIA values of 2.05‰ and 1.94‰ respectively, the dissolution resistant species *G. dubia* (*dutertrei*) and *G. menardii* had GIA values of 1.37‰ and 0.75‰ respectively. This trend has been shown to persist through time by Lidz *et al.*[46] who found that *G. tumida*, *G. truncatulinoides* and *G. crassaformis* showed lower GIA values compared with dissolution susceptible species (*G. sacculifer*, *H. pelagica*, *O. universa* and *G. conglobatus*).

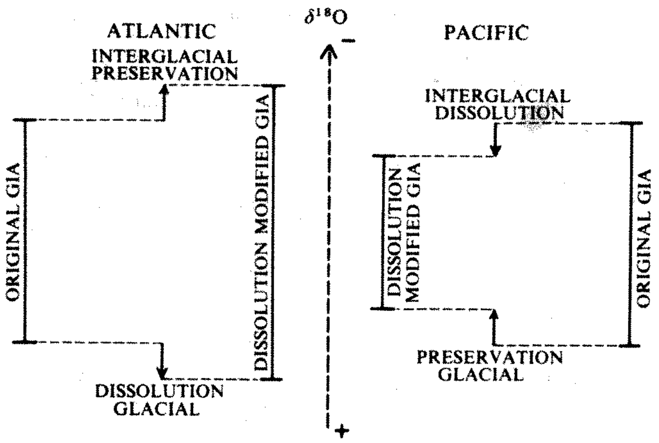

Fig. 2 Schematic presentation of modifications in the glacial–interglacial amplitude (GIA) of $\delta^{18}O$ curves caused by dissolution cycles in the Atlantic and the Pacific Oceans. The original GIA is that which would have been recorded in a well preserved core (above the lysocline) if dissolution–preservation cycles would not have existed. Note that $CaCO_3$ dissolution cycles amplify the GIA in the Atlantic while they reduce the GIA in the Pacific.

Discussion

The observations discussed above can be interpreted to show that processes other than dissolution were in operation. Emiliani[5,6,44], Shackleton and Opdyke[2], Lidz *et al.*[46], and Berger and Gardner[41], suggested that the higher GIA in the Atlantic is a result of significant temperature fluctuations between the glacials and interglacials. Vertical migration of planktonic foraminifera combined with temperature changes between glacials and interglacials can potentially account for the differences between benthonic and planktonic foraminifera as well as the differences between shallow and deep dwelling planktonics. Quantitative estimates of palaeotemperatures derived from microfossil distribution in deep sea cores support the idea that small glacial–interglacial temperature fluctuations indeed occurred in the Atlantic[44,47,48]. However, using the same quantitative techniques, other studies have shown that similar or even larger temperature fluctuations occurred in the Pacific[49]. Therefore, micropalaeontologically derived palaeotemperatures cannot explain the difference in the GIA between the two oceans.

Recently Shackleton[4] has shown that the GIA recorded by benthonic foraminifera is at least 1.65‰, and this value is believed to represent the change in $\delta^{18}O$ of ocean water due to ice accumulation and melting. In comparison, *G. sacculifer* in the Pacific shows an average GIA of 1.13‰ (based on 13 cores in Table 2). If this difference is interpreted in terms of temperature changes, it implies that *G. sacculifer* in the Pacific lived in warmer water during the glacial and colder water during the interglacial. While this is not impossible, it is more likely that the reduced GIA of *G. sacculifer* is caused by dissolution. If dissolution does reduce the GIA of planktonic foraminifera in the Pacific, it must also increase the GIA in the Atlantic. Indeed, *G. sacculifer* in the Atlantic shows an average GIA of 1.82‰ (based on 14 cores, Table 2). Therefore, there is no need to evoke cooling and warming of Atlantic surface water to explain the differences in the GIA between the two oceans.

Finally, effects of bioturbation on the isotopic record must be considered. It has been recognised since the pioneer work of Emiliani[5,6] that bioturbation can blur the isotopic record and thus reduce the GIA[4–6,50]. Because biogenic mixing affects only the upper few centimetres, cores with low accumulation rates are expected to show a lower GIA than those with high accumulation rates. Shackleton[4] showed that this is indeed the case in a few selected cores from the Atlantic and the Pacific. However, in these studies no differentiation was made between planktonic and benthonic foraminifera. Peng *et al.*[51] suggested a model to account for the effects of bioturbation on the GIA. Their model matches their data for benthonic foraminifera much better than it does for planktonic foraminifera (see Fig. 4 in ref. 51). Most of their data points for planktonic foraminifera are from the Pacific and they display a lower GIA than expected if bioturbation was the main factor in operation. Figure 1 shows the GIA versus sedimentation rate for all the data recently available for planktonic foraminifera (see also Table 2). In addition, the predicted GIA has been reproduced using the Peng *et al.*[51] model. It is clear that most Pacific data points fall below the predicted GIA and most Atlantic data points fall above the predicted GIA. Thus, although the effects of bioturbation are certainly important, dissolution certainly adds a significant modification to the isotopic record that cannot be ignored. Perhaps when techniques to 'unmix' the past record[51,52] are improved, better assessment of dissolution effects will be possible.

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Aminostratigraphy of UK Pleistocene deposits

Gifford H. Miller*, John T. Hollin & John T. Andrews*

Institute of Arctic and Alpine Research, University of Colorado, Boulder, Colorado 80309
* Department of Geological Sciences, University of Colorado, Boulder, Colorado 80309

Ratios of D-*alloisoleucine to* L-*isoleucine have been determined on freshwater molluscs from 13 interglacial sites in South East England and on marine molluscs and foraminifera from 10 Pleistocene and Upper Pliocene Crag sites in East Anglia. We can distinguish the Ipswichian, then either one or two interglacials that may be younger than the classical Hoxnian, then the Pleistocene Crags, then the Pliocene Coralline Crag.*

AMINO ACID reactions derived from protein degradation in molluscan fossils have received considerable attention as a means of correlating and dating Pleistocene deposits[1-5]. Although several reactions are potential chronological indices, in this study we have concentrated on the isoleucine epimerisation reaction. Over geological time, isoleucine, initially present in shell protein exclusively in the L configuration, epimerises to its non-protein diastereomer D-alloisoleucine. The proportion of the two acids, expressed as the allo/iso ratio, increases to an equilibrium value of 1.30±0.05. The rate of the epimerisation reaction is most sensitive to temperature; other potentially influential environmental parameters such as pH and leaching are effectively excluded by the integrity and natural buffering effects of the carbonate matrix. The activation energy for the isoleucine epimerisation reaction in carbonate fossils is ~ 29.0 kcal mol^{-1} (ref. 6). This corresponds to an approximate doubling of the apparent age for every 4 °C temperature rise in the range between 0 and 20 °C. Consequently, absolute age assessment requires a precise estimate of the integrated thermal history since shell deposition for each site and an accurate kinetic model. It is, however, possible to circumvent the thermal term by analysing samples from a limited geographic region over which the temperature fluctuations, while unknown, can be assumed to have affected the region uniformly. In such a region, amino acid ratios may be used directly as correlation and relative age indices (aminostratigraphy[7]). We favour this approach as it requires a minimum of assumptions and yields the least ambiguous results. Another potential source of error results from the non-linear relationship between reaction rate and temperature. Samples lying close to the surface for a substantial portion of their burial history may yield anomalously high ratios due to their greater seasonal and diurnal temperature ranges. We prefer to analyse samples collected from at least 1 m depth.

Interglacial sites and amino acid analyses

For our work on interglacial deposits from the Thames estuary and adjacent sites we chose the freshwater mollusc *Corbicula fluminalis*. This shell is abundant in many English interglacial sites[8]. It does not occur in England today, but the genus is widely distributed in the Old World and is currently invading the US[9]. Most of our sites were largely worked out in the last century, and most of our samples came from the collections in the British Museum (Natural History). We stress that the precise provenance of these samples is unknown. Without more historical research, the BMNH localities should be preceded by the words 'somewhere at'. However, this study was exploratory and for this purpose our samples have served very well.

From each site we have analysed three or more valves of *C. fluminalis* (when possible we select right values to avoid analysing the same individual twice). Amino acid residues in both the free and total (free plus peptide-bound) fractions are determined on an automated ion-exchange amino acid analyser[7]. Monospecific samples were chosen to avoid problems with the species dependency of racemisation rates.

Table 1 shows the isoleucine epimerisation ratios in the total amino acid fraction for all *Corbicula* analysed. A certain amount of scatter in the amino acid ratios is expected; Miller and Hare[9] suggest this should amount to less than 10–15% of the mean. The mean value is considered representative of the deposit for

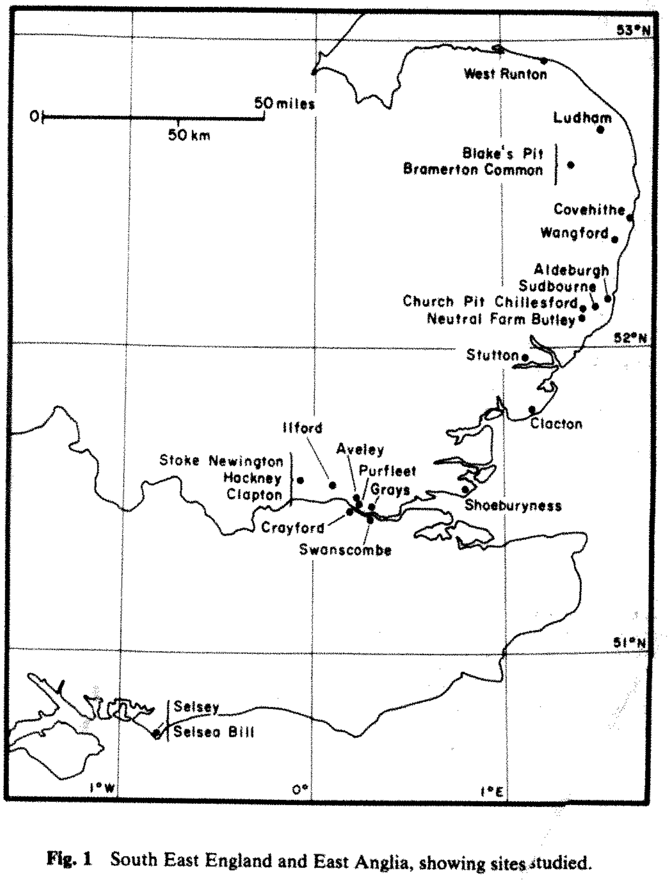

Fig. 1 South East England and East Anglia, showing sites studied.

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Table 1 Isoleucine epimerisation in *Corbicula fluminalis*

Lab ID		Locality	Group	Isoleucine epimerisation				Asp/Glu*
				Indiv.	Lowest	$\bar{x}, \sigma$	σ%	
812	A	Arkansas River†	1	0.020				3.3
		(USA)		0.021	0.020	0.020±0.001	5	2.6
811	A	Aveley‡	2	0.20				3.5
	B			0.16				4.6
	C			0.20	0.16	0.19±0.023	12	3.8
708	A	Stutton§	2	0.18				7.7
	B			0.17				7.7
	C			0.18				6.2
	D			0.24				2.6
	E			0.17				8.5
	F			0.19				7.4
	G			0.21				6.9
	H			0.17	0.17	0.19±0.024	13	6.8
701	A	Crayford‖	2	0.18				8.4
	B	(Norris's Pit)		0.21				7.6
	C			0.26				4.4
	D			0.22				—
	E			0.19	0.18	0.21±0.031	15	8.3
810	A	Clacton§	2	0.19	0.19	0.19	—	5.7
700	A	Ilford§	2	0.21				7.4
	B	(Uphall Pit)		0.26				4.7
	C			0.21				7.9
	D			0.28				5.0
	E			0.19	0.19	0.23±0.038	17	7.6
707	A	Shoeburyness§	2	0.19				>3.5
	B			0.26				3.9
	C			0.25	0.19	0.23±0.038	17	4.6
699	A	Selsey§	2 or 3	0.30				3.4
	B			0.21				5.5
	C			0.31				3.1
	D–G			0.28	0.21	0.28±0.045	16	3.9
705	A	Hackney§	2 or 3	0.24				3.7
	B			0.26				4.7
	C			0.31	0.24	0.27±0.036	13	4.0
706	A	Clapton§	3	0.29				3.9
	B			0.27				>3.4
	C			0.29	0.27	0.28±0.012	4	4.7
702	A	Grays§	3	0.29				3.8
	B			0.28				5.0
	C			0.33				3.9
	D			0.34	0.28	0.31±0.029	9	3.0
703	A	Swanscombe‖	3	0.30				6.6
	B	(Dierden's Pit)		0.29				5.8
	C			0.29	0.29	0.30±0.017	6	—
704	A	Stoke Newington§	3	0.29				3.5
	B			0.30				3.4
	C			0.34	0.29	0.31±0.026	8	2.8
709	A	Purfleet¶	4	0.33				—
	B	(shelly channel)		0.39				3.9
	C			0.34	0.33	0.35±0.032	9	3.3
710	A	Purfleet ✱	4	0.34				4.0
	B	(laminated beds)		0.34				4.1
	C			0.37	0.34	0.36±0.015	4	2.7
711	A	Wangford**	5	0.74				3.0
	B	(Hill Farm, Old Pit)		0.78				3.7
	C			0.75	0.74	0.76±0.021	4	4.1

Epimerisation was determined as the ratio of D-alloisoleucine to L-isoleucine in the total amino acid assemblage after conventional acid hydrolysis.

* We have noted a correlation between the ratio of aspartic acid to glutamic acid (Asp/Glu) and the isoleucine epimerisation ratio within shells of Group 2. *Corbicula* with low Asp/Glu values have high allo/iso ratios. In the Stutton collection analyses 708 D-H were made on different portions of a single valve. 708 D, the hinge area, yielded an allo/iso ratio substantially above the other portions (centre, central, anterior and posterior growing edges; E–H respectively). Similarly, the Asp/Glu ratio in 708 D is less than half the value of the other portions. The cause of such differences within a single valve is not readily apparent, but may be related to the calicification process. Weiner[37] has recently demonstrated a link between aspartic acid and calcium in mollusc shells. We have refrained from analysing the hinge area in other specimens to minimise the intravalve variation.

† *Corbicula* sp. from Russellville, Arkansas, provided by Dr L. R. Kraemer.

‡ Sample C from Aveley collected by Mr G. R. Ward of the Passmore Edwards Museum, from Hollin's (ref. 12) Unit 3. Samples A and B, from BMNH collections, are probably from the same unit.

§ Samples from BMNH collections, via Mr J. Cooper. The Stutton, Ilford, Shoeburyness, Grays, and probably the Clacton samples are from the Kennard collection, Selsey from the Davis collection, Hackney from the Evans collection and Stoke Newington from the Smith collection. The origin of the Clapton shells is uncertain.

‖ Collector Dr M. P. Kerney. ¶ Collector J. T. H. ✱ Collector Mr T. J. Allen. ** Collector Mr P. Cambridge.

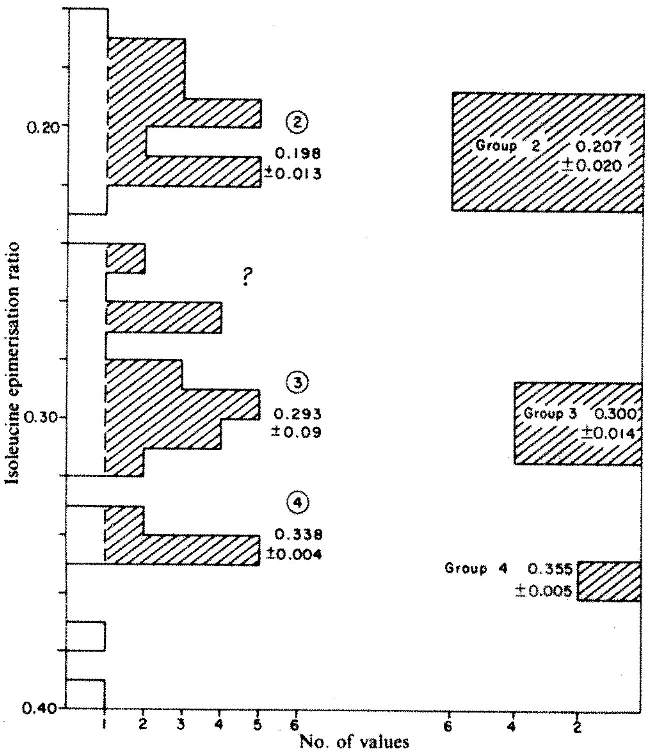

Fig. 2 Frequency distribution of individual isoleucine epimerisation (allo/iso) ratios in *Corbicula* from interglacial sites in SE England. Only ratios with more than one individual determination were considered significant in defining the three clusters on the left. Means and standard deviations from Groups 2–4 in Table 1 are on right for comparison.

collections with a standard deviation of 15% or less. Greater deviations mean that one or more determinations are suspect. As in most Quaternary fluvial and shallow marine deposits, some samples may be reworked[10]. Hence, it may be more realistic to consider only the lowest ratio when comparing sites (Table 1). However, whether we consider the mean, or the lowest allo/iso ratios, five distinct groups are apparent in Table 1, with the only overlap being at Selsey and Hackney.

There is another, independent technique of evaluating the amino acid data. We can start with the assumption that the stratigraphy of most sites is not sufficiently documented to demonstrate the contemporaneity of each valve with a specific stratum. Consequently we may simply have a collection of *Corbicula* from an unknown array of interglacial ages. In Fig. 2 we have plotted the distribution of individual allo/iso determinations between 0.15 and 0.40 (Groups 2, 3 and 4). The distribution is clearly non-random and three prominent clusters are apparent, with a possible additional cluster between allo/iso 0.24 and 0.26. The means and deviations for these clusters compare favourably with the means of Groups 2, 3 and 4 in Table 1 (see Fig. 2). Whether the allo/iso ratios are plotted by minimum or mean values for each collection, or simply as a composite of all values, five groups are clearly defined, with few ambiguous samples.

Amino acid groups and British Standard Stages

Group 1, a modern sample from Arkansas, is separated by a considerable interval from Group 2. Most of the Group 2 sites have been assigned by most workers to the last, Ipswichian, interglacial in the British Standard Stages[11]. Comprehensive references to the sites at Aveley, Sutton, Crayford and Ilford are given in ref. 12. The location of the *Corbicula* site at Shoeburyness is uncertain[13–15]. The pollen at Selsey is typically Ipswichian[16], and perhaps the higher ratios there represent a temperature effect on the shells, from either their southerly position or their shallow burial on the foreshore. However, note that Baden-Powell[17] assigned some marine shells in this area to his 'Tyrrhenian', last-but-one interglacial. The only anomalous result in Group 2 is from Clacton, which is generally assigned to the Hoxnian interglacial in the Standard Stages. Only a few *Corbicula* have been reported from Clacton[18]. The BMNH Kennard collection contains only a few valves, and we agreed to analyse one juvenile. Our result is so precisely Ipswichian that it is unlikely to represent a failure of our method. Either the collection is mixed, or perhaps the 3 km extent of the Clacton deposits[18,19] includes a patch of Ipswichian material. In another attempt to relate the amino acid ratios to a Hoxnian pollen site, Cooper was able to supply one *Macoma* sp. marine shell from Selsea Bill and two *Macoma* from Clacton. In this case the Clacton shells were clearly older. Note from the Selsey ratios in Tables 1 and 2 that *Macoma* racemises more rapidly than *Corbicula*.

Most of our Group 3 sites have been assigned at some time to a last-but-one interglacial. However, none of them has yielded a diagnostic pollen diagram, and their assignment to the Hoxnian Stage is far from certain. The Hackney, Clapton and Stoke Newington samples are presumably all from the Hackney

Table 2 Isoleucine epimerisation in molluscs from Crag deposits

Unit	Site	No. of valves analysed	*Mya* sp. $\bar{x}$, σ	No. of valves analysed	*Macoma* sp. $\bar{x}$, σ
Ipswichian	Selsea Bill, Sussex*			1	0.33 (0.33)
Hoxnian	Clacton, Essex*			2	0.58 ± 0.03 (0.54)
Weybourne Crag	West Runton†	2	0.86 ± 0.028 (0.84)	2	0.90 ± 0.02 (0.83)
Norwich Crag Formation	Wangford near Hill farm†	3	0.89 ± 0.043 (0.84)		
	Covehithe†	3	0.89 ± 0.025 (0.84)		
	Blake's Pit†	3	0.96 ± 0.062 (0.91)	3	0.83 ± 0.044 (0.80)
	Bramerton Common†	2	1.02 ± 0.035 (0.99)	2	0.92 ± 0.02 (0.91)
	Church Pit‡ (Chillesford Crag)	2	1.08 ± (1.08)		
	Church Pit‡ (*Scrobicularia* Crag)	2	0.94 ± (0.94)	3	0.94 ± (0.94)
	Aldeburgh Pit†			3	1.02 ± 0.22 (0.83)
Red Crag Formation	Neutral Farm†‡	3	0.97 ± 0.098 (0.86)	3	0.71 ± 0.01 (0.67)
Coralline Crag	Sudbourne Park Pit‡	3	1.20 ± 0.02 (1.18)		

Epimerisation was measured as the ratio of D-alloisoleucine to L-isoleucine in the total acid hydrolysate. Values are mean ± s.d. of determinations on three individual valves when possible. Numbers in parentheses are lowest ratios.

* From BMNH collection, from J. Cooper.

† From P. Cambridge.

‡ Collector J. T. A.

Table 3 Isoleucine epimerisation in the foraminifera *Elphidiella hannai* from the Ludham Pilot Bore (Fig. 1)

Sample ID	Depth (m, O.D.)	Pollen zone	Foram. zone	Stage	Ratios
LP9	−11.7 to −13.1	L_p4b/4c	L_f6	Bav./Pre-Past.	0.79 0.81
LP17	−25.1 to −26.5	L_p1b/2	L_f3	Thur.-Lud. (early Norw. Crag)	0.81 0.83
LP20	−28.8 to −31.9	L_p1b	L_f2	Upper Lud. (earliest Norw. Crag)	0.90 1.06

Samples provided by B. M. Funnell, University of East Anglia, and prepared by A. R. Nelson, Dalhousie University. Each determination included several foraminifera tests. For a discussion of the stages here, see ref. 38.

London Borough sites exposed in the last century and reviewed by Wymer (ref. 20, p. 297–306). Hackney L. B. covers an area more than 3 km across, with elevations from 3 to above 30 m O.D., and it probably includes deposits of more than one age (ref. 20, pp. 317–319; ref. 27). The relatively low ratios from Hackney suggest the possibility of an interglacial stage or substage intermediate between Groups 2 and 3 in age, represented perhaps by the cluster of ratios between 0.24 and 0.26 in Fig. 2. The Grays shells are separated in the BMNH collections from those from West Thurrock, so are almost certainly from Little Thurrock[12]. Traditionally[18,20,21–23], the mammals at Little Thurrock have been assigned to a last-but-one interglacial, although West[24] and Hollin[12] thought that the polleniferous brickearth there was more likely to be Ipswichian than Hoxnian. The *Corbicula* from Little Thurrock must come from below 15 m O.D. (ref. 12). The *Corbicula* from our site at Dierden's Pit, just across the river at Swanscombe, are correlated by Kerney[25] with those reported from the Middle Gravels of the nearby Barnfield Pit, at about 27 m O.D. (ref. 20, pp. 332–354; ref. 26). *Corbicula* is notably absent from the underlying Lower Loam and Lower Gravel at Barnfield Pit[25], at an elevation intermediate between the Middle Gravels and Little Thurrock. This absence reinforces Hubbard's suggestion[27], that the Middle Gravels may be from a separate, younger interglacial than the Lower Loam and Lower Gravel. And the molluscs in the Lower Loam[25] and the archaeology from the Lower Gravel[20] resemble those at Clacton, where the pollen resembles that in the type site of the Hoxnian interglacial at Hoxne (Turner, in ref. 25).

Our Group 4 ratios come from the large interglacial site at Purfleet. Allen's[28] samples were collected 40 cm above the base of the laminated beds (Hollin's[12] Unit 2) in Greenlands north face. The ratios from the shelly channel (Unit 3) suggest that this is from the same interglacial. Hollin thought that the laminated beds were most probably Ipswichian, but very possibly older; and Allen thought that they might be from an interglacial between the Ipswichian and Hoxnian. The amino acid ratios suggest that they are at least as old as Dierden's Pit and the Middle Gravels at Swanscombe. This now fits in well with the conclusions of Palmer[29], that the archaeology from the overlying gravel and sand (Unit 4) at Purfleet resembles that in the Middle Gravels. Like that at Little Thurrock, the pollen in the laminated beds at Purfleet appears to be from the first half of an interglacial[12]. There is some overlap in the ratios at these two sites, so perhaps this is the Group 3 interglacial; it is unlikely to be the Hoxnian, since *Corbicula* is not recorded in the first half of the interglacial at Clacton[25]. And following the Clacton disappointment above, we have failed to locate any more *Corbicula* from a Hoxnian pollen site; the time relationship between our Groups 3 and 4 and the Hoxnian will have to be determined in some future campaign with some other species.

The Group 5 ratios come from the old pit at Hill Farm Wangford, in the Crag deposits, and provide a link with the marine, *Mya* sp. ratios below. Funnell and West (ref. 30, p. 253–254) discuss whether the 'Chillesford pollen assemblage' at Wangford is Pastonian (the interglacial before the Cromerian) or much older. Our results favour the latter.

Crag deposits of East Anglia

We report here on our efforts to stratify and correlate Crag deposits on the basis of the isoleucine epimerisation reaction in shells of the marine genera *Mya* and *Macoma* (Table 2). Our stratigraphic terminology follows Funnell and West[30]. We are able to link the Crag deposits to the interglacial units by comparing allo/iso ratios in *Corbicula* and *Mya* from Wangford (Tables 1 and 2; 0.76 and 0.89 respectively). The *Mya* values are somewhat above those in *Corbicula*, suggesting a more rapid epimerisation in the former. We have little faith in the *Macoma* results from the Crags, first because in this case we have no link with *Corbicula*, and second because most *Macoma* yielded anomalously low total amino acid concentrations.

Individual allo/iso ratios in *Mya* from the Crag deposits range from 0.89 to 1.25. This result indicates that epimerisation has not reached equilibrium for most sites, in spite of their age. The *Mya* ratios from the Crag units define only two broad groups; a younger one that includes the Red Crag Formation, Norwich Crag Formation and Weybourne Crag, and a second, older one

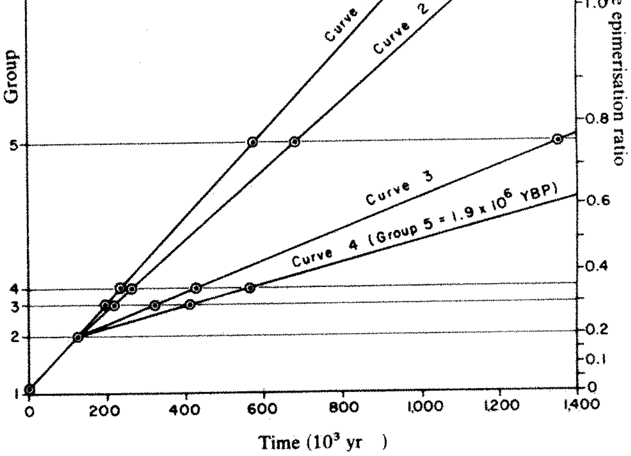

Fig. 3 Possible ages of Groups 3–5, assuming Group 2 is from 124,000 BP. Curve 1 assumes that reversible first order kinetics are followed exactly, Curve 2 that Group 3 is from 210,000 BP, Curve 3 that Group 3 is from 310,000 BP and Group 4 that Group 3 is from 410,000 BP.

consisting of the Coralline Crag. Shell fragments of *Mya* or *Arctica* collected from the Sudbourne Park Pit near Orford are from the lower shelly sands of the Coralline Crag. Allo/iso ratios are close to equilibrium, indicating that these Pliocene deposits are at the limit of the amino acid method for UK deposits. Within the younger group, average ratios range from 0.86 to 1.08. The Red Crag Formation is the oldest unit within this group and represents the basal deposits of the British Pleistocene succession. *Mya* from the Neutral Farm Pit of the Red Crag Formation have allo/iso ratios close to 1.0, significantly lower than those in the underlying Coralline Crag. Ratios in *Mya* from units within the Norwich Crag Formation range between 0.89 and 1.08, but are not always in correct stratigraphic order. Analyses of additional shell material should allow us to determine whether these discrepancies represent noise in the method or reflect reworking of shells from older units. The amino acid data suggest that the Weybourne Crag at West Runton is similar in age to the younger members of the Norwich Crag Formation.

Late in this study we were able to obtain foraminifera from the Ludham Pilot Bore in the Crag deposits. The epimerisation data (Table 3) are in the correct stratigraphic order and are similar to ratios in marine molluscs from correlative units.

Future work on the Crags of eastern England, especially if that work is concerned with the question of chrono-correlation, means analysing rather large numbers of samples. How large? If we wish to distinguish between the different Crag units, and if shells in these units differ in allo/iso ratios by 0.04, we can use our data to calculate that the number of runs required to discriminate between the units is equal to:

$$N = 4\sigma^2/L^2$$

where σ^2 is the variance of the population, and L is the limit we wish to apply (in our case, 0.04). If we set $\sigma^2 = \pm 0.1$ (a conservative figure) then we need 25 runs to be able to distinguish between two populations at the 95% confidence level. If $\sigma^2 = \pm 0.06$ then nine samples would be sufficient.

Discussion

The ratios above are generally in the correct chronostratigraphic order, and we discuss now how they may correlate with the worldwide marine oxygen isotope stratigraphy[31] and with an absolute time scale[32]. In Group 2, the presumably tidal[12,28] laminations at Crayford, the maritime herbs at Ilford, and the apparent level of the old floodplain at Aveley all suggest an early Ipswichian sea level of roughly 7 m (ref. 12: note that the possibility of the laminated beds at Little Thurrock and Purfleet being pre-Ipswichian was allowed for in that paper). In turn, this suggests a correlation with the worldwide high sea stand at 124,000 BP[33] and thereby with isotope Stage 5e[34]. A U-series date of 125,000 BP has been obtained on a bone from Stutton[35].

On the assumption that Group 2 (mean ratio 0.207) dates from 124,000 BP, we can make some estimate of the ages of the older groups. However, conversion of allo/iso ratios into absolute ages is not straightforward. The isoleucine epimerisation reaction does not follow first-order reversible kinetics in carbonate fossils. Shell amino acids, initially bound in high molecular weight peptide chains, are slowly released in the free state over geological time but are retained within the carbonate matrix. The epimerisation rate differs for free and bound isoleucine [relative reaction rates as follows: terminal (C or N) $\gg$ internal $\approx$ free][36]. Because the proportion of free isoleucine increases in older samples, there is a general reduction in the epimerisation rate with increasing sample age. First order kinetics may eventually be approximated, but at no point does the reaction rate increase. Hence, temporal extrapolations from younger (last interglacial) to older samples will yield minimum ages.

In Fig. 3, we have plotted the allo/iso ratios

$$\ln\left(\frac{1+\mathrm{D/L}}{1-0.77(\mathrm{D/L})}\right) \quad \text{against time}^6$$

Curve 1 assumes shells in Group 2 are 124,000 yr old and that the isoleucine epimerisation reaction in *Corbicula* follows first order reversible kinetics exactly. The age predicted for Group 3 (190,000 BP) is a little low for the pre-Ipswichian interglacial Stage 7 (ref. 32). If we fix the age of Group 3 at 210,000 BP (roughly the mid-point of Stage 7), the age of Group 5 becomes $\geq$680,000 BP (curve 2). Fixing Group 3 at 310,000 BP (roughly the mid-point of the next interglacial, Stage 9), yields a Group 5 age of $\geq 1.35 \times 10^6$ BP (curve 3). Fixing Group 3 at 410,000 BP (an early estimate for the age of Stage 11), yields a Group 5 age of 1.9×10^6 BP (curve 4), which seems improbably old for the Norwich Crag.

We conclude, therefore, that our Group 3 probably belongs to isotope Stage 7 or 9. Unfortunately, we do not feel that the available geological evidence allows us to choose between these possibilities as yet. If we assign Group 3 ratios to Stage 7, extrapolation from Table 2 and Fig. 3 suggests an age for the younger Crag group of 0.7 to 1.2×10^6 BP and for the Coralline Crag of 1.9×10^6 BP. If we assign Group 3 to Stage 9, these ages become 1.0 to 1.8×10^6 BP and $\sim 3.0 \times 10^6$ BP. Especially valuable now would be a UK Pleistocene section with a recognisable palaeomagnetic reversal bracketed by shells.

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Direct expression in *Escherichia coli* of a DNA sequence coding for human growth hormone

David V. Goeddel*, Herbert L. Heyneker*, Toyohara Hozumi†, Rene Arentzen†, Keiichi Itakura†, Daniel G. Yansura*, Michael J. Ross*, Giuseppe Miozzari*, Roberto Crea‡ & Peter H. Seeburg*

Divisions of Molecular Biology* and Organic Chemistry‡, Genentech Inc., 460 Point San Bruno Boulevard, South San Francisco, California 94080
† Division of Biology, City of Hope National Medical Center, Duarte, California 91010

DNA coding for human growth hormone was constructed by using chemically synthesised DNA in conjunction with enzymatically prepared cDNA. This 'hybrid' gene was expressed in Escherichia coli *under the control of the* lac *promoter. A polypeptide was produced having the size and immunological properties characteristic of mature human growth hormone.*

HUMAN GROWTH HORMONE (HGH) is a protein of 191 amino acids which is synthesised in the anterior lobe of the pituitary. Growth in hypopituitary dwarfs, whose small stature is due to a deficiency of HGH, can be restored during childhood by administration of this hormone[1]. In addition, HGH may prove effective in the treatment of a variety of ailments, including bone fractures, skin burns and bleeding ulcers[2]. As growth hormone is species specific, human cadavers have been the only source of HGH.

The primary translation product of growth hormone mRNA is a precursor protein consisting of a signal peptide attached to the N-terminus of growth hormone[3]. Such signal or 'pre' sequences are characteristic of secreted proteins. In the case of rat growth hormone (RGH), DNA sequencing of cDNA prepared from RGH mRNA has identified the 26 amino acid residues of the pre-sequence[4]. From information derived from the cDNA sequence of human growth hormone[5] we have designed and constructed a bacterial plasmid which instructs the synthesis of substantial quantities of mature HGH in a microbial cell.

HGH gene assembly strategy

The general approach used for the bacterial synthesis of HGH involves a combination of the cloning of the complementary DNA (cDNA) prepared from pituitary mRNA with the cloning of chemically synthesised DNA. The specific strategy was based on the known restriction endonuclease pattern of HGH cDNA[5]. *Hae*III sites are present in the 3′ noncoding region and in the sequence coding for amino acids 23 and 24 of HGH. Treatment of double stranded (ds) HGH cDNA with *Hae*III gives a DNA fragment of 551 base pairs which includes coding sequences for amino acids 24–191 of HGH. We planned to clone this cDNA fragment and to clone separately a chemically synthesised DNA 'adaptor' fragment containing an ATG initiation codon and coding sequences for residues 1–23 of HGH. These two DNA fragments would be combined to form a synthetic–natural 'hybrid' gene. When inserted into a plasmid downstream from a uitable bacterial promoter and ribosome binding site, this gene uld be expected to direct the synthesis of fMet-HGH. The fact [illegible] most bacterial proteins do not contain N-terminal ionine residues suggests that the fMet should be efficiently ed, resulting in the direct expression of HGH.

[illegible] are several advantages of this approach over con- [illegible] expression methods which utilise either chemically [illegible] DNA or cDNA exclusively. The cDNA approach has been used to express several fused proteins (β-lactamase–rat proinsulin[6], β-lactamase–rat pre-growth hormone[7] and β-galactosidase–chicken ovalbumin[8,9]) in *Escherichia coli* which still retain antigenic activity, but which cannot be easily processed to give the natural gene product. However, the cloning of mouse dihydrofolate reductase cDNA into G-tailed, *Pst*I-cleaved pBR322 resulted in the expression of a protein which appeared to be dihydrofolate reductase[10]. This protein could have been derived from the proteolytic cleavage of a β-lactamase–dihydrofolate reductase fused protein or from the direct initiation of translation at the dihydrofolate reductase ATG (AUG) start codon. Neither of these possibilities could be relied on for HGH expression. *In vivo* proteolytic cleavage of a fused bacterially produced protein to give HGH is highly unlikely, and initiation of translation at an HGH start codon would be expected to yield the pre-hormone containing an extra 26 amino acids. The alternative approach of complete chemical synthesis of genes, although shown to be extremely effective for the bacterial production of the human peptides somatostatin[11] and insulin[12], would be very time consuming for a polypeptide as large as HGH.

Construction and cloning of plasmids containing HGH DNA sequences

The plan for the chemical synthesis of DNA designed to code for the first 24 amino acids of HGH is shown in Fig. 1*a*. To facilitate joining to HGH cDNA, the synthetic plan incorporates the same *Hae*III restriction site at amino acid residues 23 and 24 that is found in the cDNA. The 12 indicated deoxyoligonucleotides (Fig. 1*a*) were synthesised by the improved phosphotriester method essentially as described previously for insulin[13]. Figure 1*b* summarises the steps involved in the assembly and cloning of these DNA fragments in pBR322 (ref. 15). Many transformants of *E. coli* 294 (ref. 16) were obtained with plasmids containing *Eco*RI–*Hin*dIII inserts of approximately the desired size. The nucleotide sequences of three of these inserts were determined. One, pHGH3, had the expected DNA sequence. The other two had a deletion of the $A \cdot T$ base pair at the third nucleotide position for the codon of amino acid 5 (Pro).

The construction of the plasmid (pHGH31) containing the *Hae*III cDNA fragment of HGH is outlined in Fig. 2. Total poly(A)-mRNA from human pituitaries was used in the synthesis of ds-cDNA by RNA-dependent DNA polymerase (reverse transcriptase) and DNA polymerase I Klenow fragment. The cDNA was treated with *Hae*III restriction endonuclease and DNA of about 550 base pairs was purified by gel electrophoresis. This DNA fraction was then 'tailed' with deoxyC residues using terminal deoxynucleotidyl transferase[10] and cloned in pBR322 DNA which had been cleaved with *Pst*I and extended with deoxyG residues. This procedure restores the *Hae*III restriction sites on the cDNA and regenerates *Pst*I sites at both ends of the insert. Approximately 200 transformants of *E. coli* χ1776 (ref. 20) were obtained from 1 ng of C-tailed cDNA. These colonies were screened for the presence of HGH

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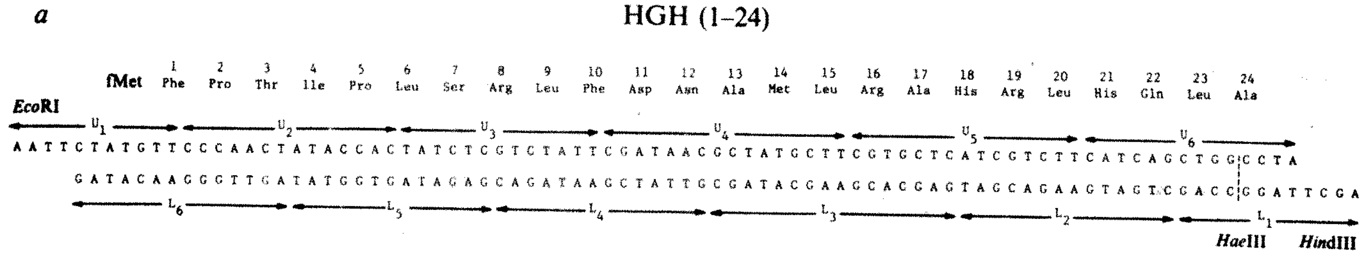
a
HGH (1–24)
fMet
1 Phe
2 Pro
3 Thr
4 Ile
5 Pro
6 Leu
7 Ser
8 Arg
9 Leu
10 Phe
11 Asp
12 Asn
13 Ala
14 Met
15 Leu
16 Arg
17 Ala
18 His
19 Arg
20 Leu
21 His
22 Gln
23 Leu
24 Ala
EcoRI
U1 U2 U3 U4 U5 U6
A A T T C T A T G T T C C C A A C T A T A C C A C T A T C T C G T C T A T T C G A T A A C G C T A T G C T T C G T G C T C A T C G T C T T C A T C A G C T G G C C T A
G A T A C A A G G G T T G A T A T G G T G A T A G A G C A G A T A A G C T A T T G C G A T A C G A A G C A C G A G T A G C A G A A G T A G T C G A C C G G A T T C G A
L6 L5 L4 L3 L2 L1
HaeIII
HindIII

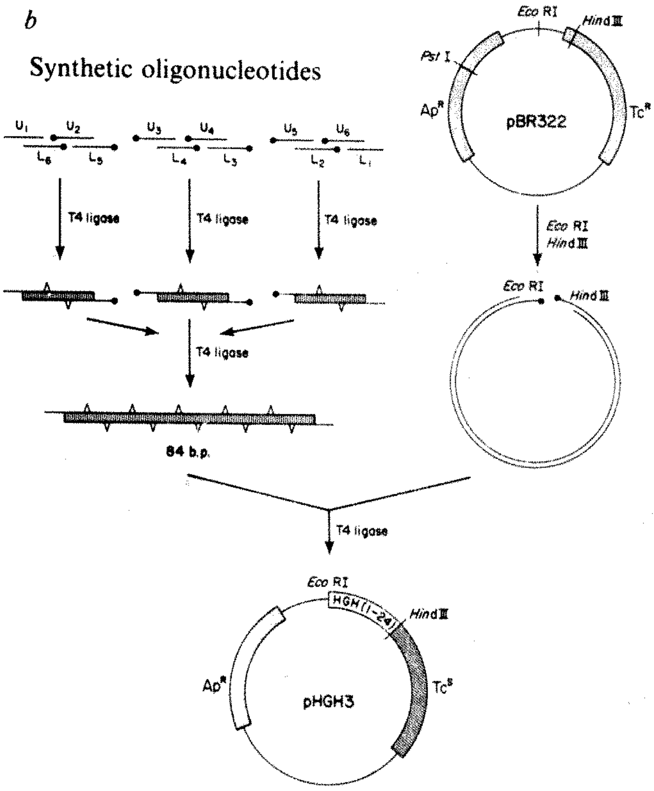

Fig. 1 Design and molecular cloning of chemically synthesised DNA coding for the first 24 amino acids of human growth hormone. *a*, The synthetic plan. The 5′ ends have single-stranded cohesive termini for the *Eco*RI and *Hin*dIII restriction endonucleases to facilitate plasmid construction. The methionine codon at the left end provides a site for initiation of translation. Twelve different oligonucleotides, varying in size from undecamer to hexadecamer, were synthesised by the improved phosophotriester method[13]. These oligonucleotides, U_1 to U_6 and L_1 to L_6, are indicated by arrows. *b*, Assembly of chemically synthesised DNA fragments and construction of plasmid pHGH3 containing a 84-base pair insert coding for HGH amino acids 1–24. Aliquots of 10 µg of U_2–U_6 and L_2–L_6 were phosphorylated using T_4 polynucleotide kinase and [γ-^{32}P]ATP (NEN) by a procedure of D.V.G.[12]. Three separate T_4 ligase-catalysed joining reactions were performed: 10 µg of 5′-OH fragment U_1 was combined with the phosphorylated U_2, L_5 and L_6; phosphorylated U_3, U_4, L_3 and L_4 were combined; and 10 µg of 5′-OH fragment L_1 was combined with the phosphorylated L_2, U_5 and U_6. These ligations were carried out at 4 °C for 6 h in 300 µl of 20 mM Tris-HCl (*p*H 7.5), 10 mM $MgCl_2$, 10 mM dithiothreitol, 0.5 mM ATP using 20 units of T_4 ligase. The three ligation mixtures were then combined, 20 units of T_4 ligase added, and the reaction allowed to proceed for 12 h at 20 °C. The mixture was ethanol-precipitated and electrophoresed on a 10% polyacrylamide gel. The band migrating at 84 base pairs (bp) was sliced from the gel and eluted[14]. Plasmid pBR322 (ref. 15) (1 µg) was treated with *Eco*RI and *Hin*dIII, the large fragment isolated by gel electrophoresis and ligated to the synthetic DNA[12]. This mixture was used to transform *E. coli* 294 (ref. 16). The *Eco*RI–*Hin*dIII insert of pHGH3 had the correct sequence as determined by the Maxam–Gilbert method[14].

sequences by the Grunstein–Hogness hybridisation procedure[21] using ^{32}P-labelled human chorionic somatomammotropin cDNA[22] as probe. Seven colonies hybridised specifically with the probe, which is nearly identical to HGH in nucleotide sequence[5]. Three had identical *Hae*III inserts of approximately 550 base pairs containing *Sma*I, *Bgl*II, *Pst*I and *Pvu*II sites, all characteristic of HGH cDNA[5]. Sequence analysis of the insert from one of these clones, pHGH31 (Fig. 3) gave only one discrepancy (CUG versus CUA codon for leucine 101) when compared with the previously determined cDNA sequence[5]. The amino acid sequence derived from the cDNA sequence differs from the published amino acid sequences of Li[23] at positions 74 (Glu versus Gln), 107 (Asp versus Asn) and 109 (Asn versus Asp). Although we cannot rule out post-transcriptional modification, the discrepancies probably stem from shortcomings of earlier protein sequencing methods in the identification of amidated amino acids.

Construction and cloning of HGH expression plasmids

To express directly HGH in *E. coli*, a plasmid (pGH6) having two *lac* promoters was constructed as follows. A 285-base pair *Eco*RI fragment containing two 95-base pair UV-5 *lac* promoter fragments separated by a 95-base pair heterologous DNA fragment was isolated from the plasmid pKB268 (ref. 24) and inserted into pBR322 at the *Eco*RI site. A plasmid (pGH1) was isolated with the promoter orientated to initiate transcription in the direction of the tetracycline resistance (tc^R) gene. The *Eco*RI site distal to the tc^R gene was destroyed by a previously outlined procedure[11].

Figure 4 reviews the steps involved in assembling the two separately cloned HGH DNA fragments. Treatment of pHGH3 with *Eco*RI and *Hae*III gives a 77-base pair fragment with an *Eco*RI cohesive end and a *Hae*III blunt end (also see Fig. 1*a*). This fragment, which codes for HGH amino acids 1–23, was isolated from a polyacrylamide gel. The plasmid pHGH31 was cleaved with *Hae*III and the 55-base pair HGH cDNA fragment isolated by gel electrophoresis. *Xma*I treatment of this fragment yielded a 512-base pair fragment coding for amino acids 24–191 of HGH with one *Hae*III blunt end and one *Xma*I sticky end. The ligation of these two fragments resulted in the formation of high molecular weight DNA. Treatment with *Sma*I (which recognises the same sequence as *Xma*I but leave flush ends) and *Eco*RI converted the ligation products to three distinct fragments: a 154-base pair dimer of the 77-base pair DNA fragment, a 1,024-base pair dimer of the 512-base pair fragment, and the desired 591-base pair DNA coding for the entire HGH sequence. This latter fragment was purified by gel electrophoresis and inserted into pGH6 between the *Eco*RI and the S_1 nuclease-treated *Hin*dIII sites. Following transformation of χ1776, the expression plasmid pHGH107 was identified by colony screening and restriction analysis as described in Fig. 3. The HGH DNA sequence of pHGH107 was verified by the Maxam–Gilbert procedure[14].

In our plan to express HGH two base pairs were included between the *Eco*RI AATT 'sticky' end and the ATG codon of the chemically synthesised portion of the HGH coding sequence (see Fig. 1). Therefore, in the plasmid pHGH107, 11 base pairs separate the *lac* AGGA ribosome binding site[26] and the ATG translational start for HGH:

$$5'\ldots\overline{\text{AGGA}}\,\text{AACAG}|\underline{\text{AATT}}\,\text{CT}\,\overline{\text{ATG}}\ldots$$
$$3'\ldots\text{TCCTTTGTCTTAA}|\text{GATAC}\ldots$$

In the naturally occurring *lac* system the separation between

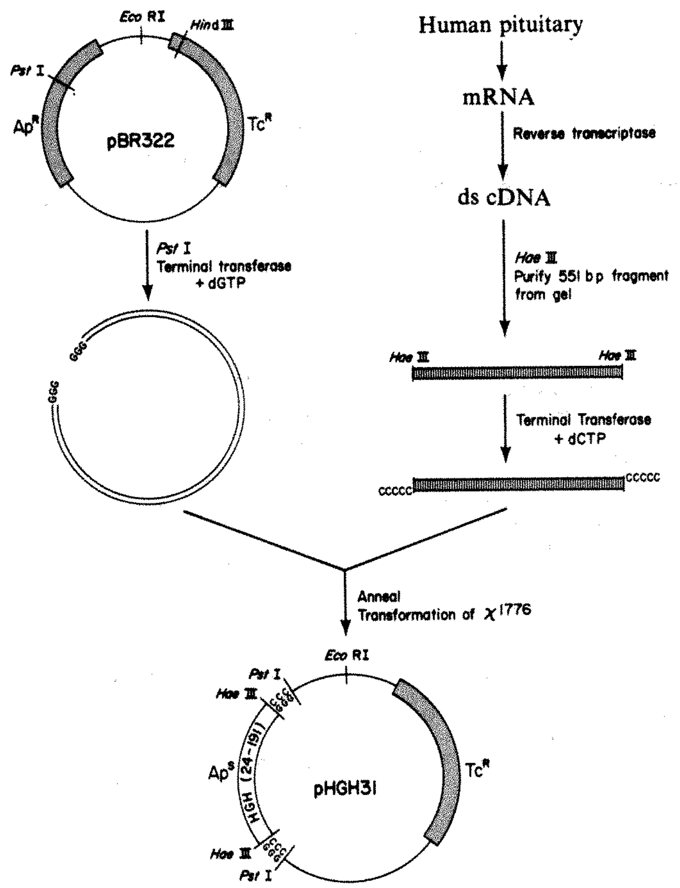

Fig. 2 Construction of a plasmid (pHGH31) containing coding sequences for amino acids 24–191 of human growth hormone. Poly(A)-mRNA was prepared from human pituitaries by a published procedure[17]. ds-cDNA (1.5 μg) was prepared from 5 μg of this RNA essentially as described by Wickens *et al.*[18], except that DNA polymerase 'Klenow fragment'[19] was substituted for DNA polymerase I in the second strand synthesis. cDNA (90 ng) was treated with *Hae*III, electrophoresed on an 8% polyacrylamide gel, and the region around 550 base pairs eluted. Approximately 1 ng of cDNA was obtained. Terminal deoxynucleotidyl transferase (TdT) was used to add approximately 20 dC residues per 3′ terminus[10]. Annealing of the dC-tailed ds-cDNA with the dG-tailed vector DNA (60 ng) was performed in 130 μl of 10 mM Tris-HCl (pH 7.5), 100 mM NaCl, 0.25 mM EDTA. The mixture was heated to 70 °C, allowed to cool slowly to 37 °C (12 h), then to 20 °C (6 h), before being used to transform χ1776 (ref. 20) by a published procedure[4].

ribosome binding site and initiation codon is 7 base pairs. The exact *lac* sequence in this region was restored as follows. The plasmid was cleaved with *Eco*RI, treated with S_1 nuclease to digest the single stranded ends, and reclosed by blunt-ended ligation. The plasmids from three individual transformants of χ1776 were analysed. In all instances the *Eco*RI site had been removed and a new *Alu*I site created in the plasmid (pHGH107–1).

$$5'\ldots\overline{\mathrm{AGGA}}\,\mathrm{AACAG}\,\vert\,\mathrm{CT}\,\overline{\mathrm{ATG}}\ldots$$
$$3'\ldots\mathrm{TCCTTTGTC}\,\vert\,\mathrm{GATAC}\ldots$$

This structure, in which the ATG codon is now seven nucleotides downstream from the ribosome binding site, might be expected to increase the efficiency with which HGH mRNA is translated.

HGH radioimmune activity in bacterial extracts

Extracts of *E. coli* χ1776 containing the expression plasmids were tested for HGH expression by a direct radioimmunoassay (RIA) (Table 1). Easily detectable amounts of HGH were produced by bacteria containing pHGH107 or the derivative of pHGH107 lacking the *Eco*RI site (pHGH107–1). Contrary to

```
        24                                                              40
        Ala Phe Asp Thr Tyr Gln Glu Phe Glu Glu Ala Tyr Ile Pro Lys Glu Gln Lys Tyr Ser Phe Leu Gln Asn Pro Gln Thr Ser
 5'...G GCC UUU GAC ACC UAC CAG GAG UUU GAA GAA GCC UAU AUC CCA AAG GAA CAG AAG UAU UCA UUC CUG CAG AAC CCC CAG ACC UCC
                                                                                            Pst I
                                60                                                                              80
Leu Cys Phe Ser Glu Ser Ile Pro Thr Pro Ser Asn Arg Glu Glu Thr Gln Gln Lys Ser Asn Leu Glu Leu Leu Arg Ile Ser Leu Leu
CUC UGU UUC UCA GAG UCU AUU CCG ACA CCC UCC AAC AGG GAG GAA ACA CAA CAG AAA UCC AAC CUA GAG CUG CUC CGC AUC UCC CUG CUG

                                                                        100
Leu Ile Gln Ser Trp Leu Glu Pro Val Gln Phe Leu Arg Ser Val Phe Ala Asn Ser Leu Val Tyr Gly Ala Ser Asp Ser Asn Val Tyr
CUC AUC CAG UCG UGG CUG GAG CCC GUG CAG UUC CUC AGG AGU GUC UUC GCC AAC AGC CUA GUG UAC GGC GCC UCU GAC AGC AAC GUC UAU

                                                        120                                                     140
Asp Leu Leu Lys Asp Leu Glu Glu Gly Ile Gln Thr Leu Met Gly Arg Leu Glu Asp Gly Ser Pro Arg Thr Gly Gln Ile Phe Lys Gln
GAC CUC CUA AAG GAC CUA GAG GAA GGC AUC CAA ACG CUG AUG GGG AGG CUG GAA GAU GGC AGC CCC CGG ACU GGG CAG AUC UUC AAG CAG
                                                                                                      Bgl II
                                                                        160
Thr Tyr Ser Lys Phe Asp Thr Asn Ser His Asn Asp Asp Ala Leu Leu Lys Asn Tyr Gly Leu Leu Tyr Cys Phe Arg Lys Asp Met Asp
ACC UAC AGC AAG UUC GAC ACA AAC UCA CAC AAC GAU GAC GCA CUA CUC AAG AAC UAC GGG CUG CUC UAC UGC UUC AGG AAG GAC AUG GAC

                                180                                         191
Lys Val Glu Thr Phe Leu Arg Ile Val Gln Cys Arg Ser Val Glu Gly Ser Cys Gly Phe Stop
AAG GUC GAG ACA UUC CUG CGC AUC GUG CAG UGC CGC UCU GUG GAG GGC AGC UGU GGC UUC UAG CUGCCCGGGUGGCAUCCCUGUGACCCCUCCCCAGU
                                                               Pvu II                  Sma I
                                                                                       Xma I

GCCUCUCCUGGCC....3'
         Hae III
```

Fig. 3 The amino acid and mRNA sequences of HGH as determined by DNA sequencing[14] of pHGH31. The nucleotide sequence from the *Sma*I site to the *Hae*III site in the 3′ noncoding region is from ref. 5, and was not determined here.

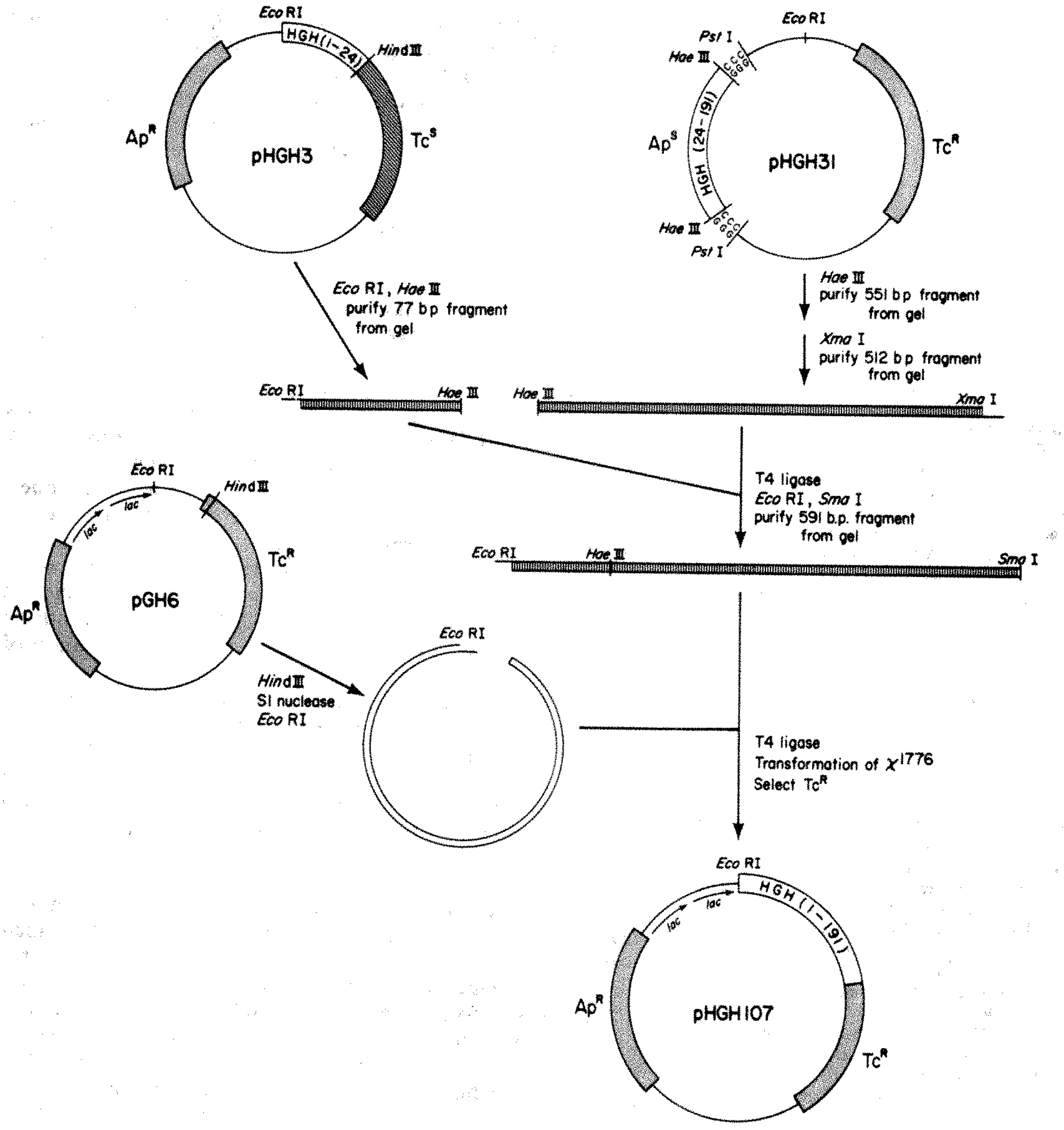

Fig. 4 Construction of a plasmid (pHGH107) for the bacterial expression of human growth hormone. pHGH3 (10 μg) was cleaved with *Eco*RI and *Hae*III restriction endonucleases and the 77-base pair fragment containing coding sequences for HGH amino acids 1–23 was isolated from an 8% polyacrylamide gel. The plasmid pHGH31 (~5 μg) was cleaved with *Hae*III. The 551-base pair HGH sequence and a co-migrating 540-base pair *Hae*III fragment of pBR322 were purified by gel electrophoresis. Subsequent treatment with *Xma*I cleaved only the HGH sequence, removing 39 base pairs from the 3′ noncoding region. The resulting 512-base pair fragment was purified from the 540-base pair pBR322 *Hae*III piece by electrophoresis on a 6% polyacrylamide gel. Samples of 0.3 μg of the 77-base pair *Eco*RI–*Hae*III fragment and 0.3 μg of the 512-base pair *Hae*III–*Xma*I fragment were polymerised with T_4 DNA ligase in a 16-μl reaction for 14 h at 4 °C. The mixture was heated at 70 °C for 5 min to inactivate the ligase, then treated with *Eco*RI (to cleave fragments which had dimerised through their *Eco*RI sites) and with *Sma*I (to cleave *Xma*I dimers), yielding a 591-base pair fragment with an *Eco*RI 'sticky' end and a *Sma*I 'blunt' end. After purification on a 6% polyacrylamide gel, approximately 30 ng of this fragment were obtained. The expression plasmid pGH6 containing tandem *lac* UV-5 promoters, was treated successively with *Hin*dIII, nuclease S_1, and *Eco*RI and purified by gel electrophoresis. This removes most of the promoter sequence of the tc^R gene, but leaves the structural gene intact (25). 50 ng of the resulting vector, which had one *Eco*RI sticky end and one blunt end, was ligated to 10 ng of the 591-base pair HGH DNA. The ligation mixture was used to transform χ1776. Colonies were selected for growth on tetracycline (12.5 g ml^{-1}). Since the tc^R promoter is no longer functional, tetracycline resistance is dependent on transcription from the *lac* promoters reading through the HGH gene sequence into the tc^R gene. Approximately 400 transformants were obtained. Colony screening by filter hybridisation[22] identified 12 colonies containing HGH sequences. The plasmids isolated from three of these colonies gave the restriction patterns expected for the correctly assembled gene when cleaved with *Hae*III, *Pvu*II and *Pst*I. The DNA sequence of one clone, pHGH107, was determined.

our expectations, the introduction of four extra base pairs of DNA into the *lac* sequence in the region between the ribosome binding site and initiation codon results in increased HGH synthesis.

To verify that HGH expression is under *lac* operon control the plasmid pHGH107 was transformed into the *E. coli* K12 strain D1210, an i^Q derivative of *E. coli* K12 HB101 which overproduces *lac* repressor[12]. The data in Table 1 demonstrate that repressor effectively blocks HGH production and that this effect can be reversed by the addition of the inducer isopropyl-β-D-thiogalactoside.

Identification of HGH from bacterial extracts

Extracts of *E. coli* K12 strain RV308 (ref. 27) containing either pHGH107 or pBR322 were electrophoresed on SDS-polyacrylamide gels. The resulting protein patterns show one clear difference (Fig. 5*a*)—an additional protein, which co-migrates with HGH isolated from pituitaries, appears in the RV308/pHGH107 extract. To achieve partial purification of HGH, an extract of a 1-litre culture of χ1776/pHGH107 was prepared and fractionated as described in Fig. 5 legend. The polyacrylamide gel electrophoresis pattern of this partially

Table 1 Radioimmunoassay of HGH in extracts from bacteria containing expression plasmids

Strain	Cell density (cells per ml)	HGH by RIA (μg per ml)	HGH copies per cell
χ1776/pHGH107	3.69×10^8	2.4	186,000
	1×10^9	1.4	39,000
χ1776/pHGH107–1	3.6×10^8	1.5	116,000
	1×10^9	0.5	14,000
χ1776/pBR322	3.6×10^8	0	0
D1210/pHGH107	3.8×10^8	2×10^{-4}	15
D1210/pHGH107 (IPTG)	3.8×10^8	1.0	7

E. coli strains containing the appropriate plasmid were gro
cell density and collected by centrifugation. Stationary
contain 10^9 cells per ml. The cell pellet was resu
Tris-HCl (*p*H 8), 50 mM EDTA, 15% sucros
lithium dodecylsulphate. After 30 min
(*p*H 7.5), 280 mM $MgCl_2$, 4 mM CaC
mixture was centrifuged for 15 min at
were analysed by radioimmunoassay us
macia). Values are the average of three in
per ml of cell culture. With HGH standar
was 0.02 ng. No activity was found in e
indicated, isopropyl-β-D-thiogalactoside (IPT
concentration of 2 mM.

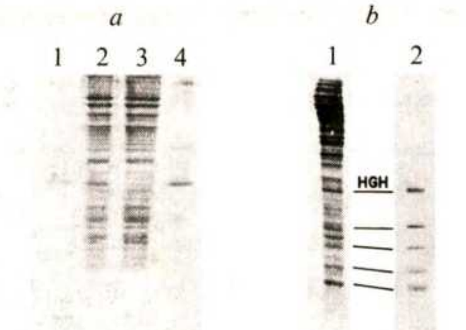

Fig. 5 Identification of HGH produced in bacteria by SDS-polyacrylamide gel electrophoresis. *a*, Protein patterns of crude extracts and partially purified HGH stained with Coomassie brilliant blue. Slot 1 contains 0.5 μg of pituitary HGH standard (Kabi), slot 2 contains a cell lysate of RV308/pHGH107, slot 3 contains a cell lysate of RV308/pBR322, slot 4 contains partially purified HGH isolated from χ1776/pHGH107. The samples were separated on a 15% polyacrylamide slab gel using the buffer system of Maizel[28] with the addition of 6 M urea. Crude lysates were prepared by growing cells in LB with 5 $\mu g\ ml^{-1}$ tetracycline followed by lysis in 2% SDS, 1% β-mercaptoethanol. The lysates were precipitated with 10 volumes of cold acetone and the pellets were redissolved in SDS sample buffer for use in gel electrophoresis. The partially purified HGH was prepared from a stationary phase culture of χ1776/pHGH107. Cells were collected and resuspended in 1/50 of their original volume in 30 mM potassium phosphate (*p*H 7.0) containing 0.05 M NaCl and lysed by sonication. Polyethylenimine (Miles, Polymin-P) was added to 0.2%. After centrifugation for 1 h at 100,000*g*, ammonium sulphate was added to the supernatant to 60% saturation. The ammonium sulphate pellet was dissolved in 2 ml 10 mM potassium phosphate (*p*H 7.0), 0.5 M NaCl and chromatographed on a Sephacryl S-200 column (2.0 × 50 cm) equilibrated in the same buffer. The radioimmune active peak (assayed with a Pharmacia Phadebus HGH kit) was pooled, the protein concentrated by ammonium sulphate precipitation, the pellet redissolved in 1/10 volume of buffer, and the solution dialysed against 10 mM potassium phosphate (*p*H 7.0), 0.5 M NaCl. Precipitated material was removed by centrifugation resulting in a HGH preparation of approximately 25% purity. *b*, Autoradiograms of ^{35}S-labelled extracts of RV308/pHGH107. Slot 1 contains a total lysate of RV308/pHGH107 labelled with $H_2{}^{35}SO_4$. Slot 2 contains an ^{35}S-labelled extract of RV308/pHGH107 precipitated with α-HGH antiserum. The major (top) band co-migrates with unlabelled HGH standard (not shown). Cultures (1 ml) of RV308/pHGH107 were grown to $A_{550} = 1$ in low sulphur medium[29] containing 0.2 $mCi\ ml^{-1}$ $H_2{}^{35}SO_4$, chased with 10 mM $MgSO_4$ for 5 min, collected and lysed using Triton X-100 and lysozyme[30]. Following DNase and RNase treatment[29], the lysate was mixed with a 10-fold excess of unlabelled RV308/pBR322 extract and diluted 1:1 into Triton immunoprecipitation buffer (0.15 M NaCl, 1% Triton X-100, 0.05 M Tris-HCl *p*H 7.5). Twenty μl of α-HGH antiserum (Kabi) was added per ml of original culture and the reaction was incubated for 12 h at 4 °C. The mixture was centrifuged and the supernatant incubated for 2 h with formaldehyde fixed *Staphylococcus* cells[31], filtered on 0.45-μm Nucleopore polycarbonate filters, washed with Triton immunoprecipitation buffer and extracted with SDS sample buffer[28]. The samples were run on a 15% slab gel containing urea and SDS as described above.

purified sample is shown in Fig. 5*a*, slot 4. The most prominent band co-migrates with HGH standard and its relative intensity is consistent with the 25% purity calculated from radioimmune activity.

In an independent attempt to confirm that the antigenically active component in the bacterial extracts co-migrates with HGH in SDS gels, we precipitated extracts from cells labelled with ^{35}S with antiserum prepared against HGH and separated the precipitated proteins by polyacrylamide gel electrophoresis. The autoradiogram of the immunoprecipitated material (Fig. 5*b*, slot 2) shows a major band, with electrophoretic mobility identical to that of HGH, as well as four smaller bands. All five of these bands are present in the unfractionated ^{35}S-labelled lysate (slot 1), whereas only the band with the same mobility as HGH can be seen in unlabelled extracts of cells grown in LB broth (Fig. 5*a*, slot 2). All five immune precipitated bands can be competed out by excess cold HGH (data not shown) and are thus antigenically related. This suggests that the four smaller HGH-related products observed in minimal media may be due to proteolytic degradation.

Additional evidence of proteolytic degradation of HGH is presented in Table 1. The levels of HGH in extracts from strains containing pHGH107 or pHGH107–1 were considerably higher in log-phase cultures than in stationary-phase cultures. Strains transformed with several other plasmids whose products are under *lac* promoter control show no comparable decrease of gene expression as a function of growth phase (unpublished results from this laboratory). It thus seems that there is some turnover of HGH in bacterial cells although we cannot rule out other explanations for the four smaller HGH-related products in the ^{35}S-labelled extracts such as premature termination of translation.

Despite the observed instability of HGH in certain growth conditions, the molecule seems to be relatively stable in log-phase cultures grown in rich media. In these conditions the amount of HGH in the cells (186,000 monomers per cell, Table 1) compares favourably with the expression level of other cloned genes using the same promoter in optimised conditions[32].

Conclusion

Using a novel combination of chemically synthesised DNA and cDNA, a recombinant *E. coli* strain has been constructed which produces HGH in large amounts. This is the first time that a human polypeptide has been directly expressed in *E. coli* in a non-precursor form. The hybrid DNA cloning techniques described as a route to the cloning and expression of HGH coding sequences in *E. coli* are generally applicable to other polypeptides which are synthesised initially as inactive precursors and later processed, or for which full length cDNA transcripts are unavailable.

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letters

On the isotropy and homogeniety of the Universe

Michael S. Turner

Astronomy and Astrophysics Center, Enrico Fermi Institute,
The University of Chicago, Chicago, Illinois 60637

It is generally believed that the Universe began from a hot big bang. Among the clues to its very early history are the present day observations that the Universe is isotropic and homogeneous (as shown by the 3 K microwave background[1] and galaxy counts[2,3]) and that there are about $10^{9\pm1}$ photons per baryon[4]. Penrose and others[5,6] argue that these two observations imply that the Universe was initially very nearly isotropic and homogeneous. Their arguments rely on absolute baryon conservation so that the baryon number of the Universe can be used as a fiducial to which the number of photons can be compared. In recently proposed grand unified theories of particle interactions, baryon number is not absolutely conserved[7,8] and thus the baryon number of the Universe is no longer a constant. It is argued here that in the absence of this fiducial the photon/baryon ratio is not a 'footprint' of the initial conditions, and therefore the initial singularity could well have been highly irregular as Misner suggested[9].

The photon/baryon ratio is also ≃ the entropy/baryon ratio (more precisely, the specific entropy/baryon ratio) as nearly all the photons in the Universe are in the 3 K background. Misner suggested that the seemingly large entropy/baryon and the remarkable isotropy and homogeneity are related. His idea is that the initial singularity, rather than being isotropic and homogeneous, was highly irregular (chaotic), and the matter was cold (small entropy per baryon). Then, dissipative processes (pair creation, neutrino viscosity, and so on) smoothed out the irregularities and in the process also generated the large entropy/baryon. This is an attractive idea for several reasons. The Universe begins from a very general initial state (rather than isotropic and homogeneous), evolves into the observed state of isotropy and homogeneity, and thereby generates the observed entropy/baryon. However, calculations by Barrow and Matzner[5] and arguments by Penrose[6] indicate that in the process of dissipating irregularities, an initially highly chaotic geometry would generate more like 10^{40} photons/baryon. Therefore, they conclude that the observed entropy/baryon of $10^{9\pm1}$ implies that the initial geometry was very nearly isotropic and homogeneous.

This reasoning relied on comparing the entropy (number of photons) to an unchanging, non-zero number (in this case, the number of baryons in the Universe). However, recent theories in particle physics suggest that baryon number is not an exactly conserved quantity. The grand unification of the strong, weak and electromagnetic forces by a gauge theory seems to require that baryon and lepton conservation be abandoned[7,8]. Unification puts quarks and leptons in the same multiplets, and therefore interactions exist which transform them into one another. In these theories baryon violating interactions are mediated by a super heavy boson (mass $m_x \gtrsim 10^{14}$ GeV) and are almost negligible at low energies (the proton is nearly stable). However, these interactions would be of roughly the same strength as all the other interactions at energies above $\sim m_x (\gtrsim 10^{14}$ GeV, temperatures above $\sim 10^{27}$ K). Several authors[10–17] have shown that these baryon non-conserving interactions along with CP violation (K^0–$\bar{K}^0$ system is known to violate CP) allow an intially hot ($T = 10^{32}$ K, the Planck temperature), baryon symmetric (net baryon number zero), isotropic ahd homogeneous Universe to evolve a net baryon excess when kT is $\sim m_x$. For reasonable values of the particle physics parameters the excess/entropy can be $\sim 10^{-9\pm1}$, and thus when the baryons and antibaryons annihilate, the Universe is left with $10^{-9\pm1}$ baryons per photon as we observe today. In addition, these grand unification theories predict a finite lifetime for the proton of $10^{31\pm1}$ years[18] which may soon be accessible to measurement. The current lower limit on the lifetime of the proton is 10^{29} yr (ref. 19).

If these ideas are correct there may no longer be a conserved, non-zero quantity to which the entropy can be compared, and therefore the entropy cannot be used as a probe of the initial geometry of the Universe. For example, the Universe could have begun from a state with highly chaotic geometry and a definite number of cold baryons; very early ($t \sim t_{\text{planck}} \sim 10^{-43}$ s, $T \sim 10^{32}$ K), dissipative processes smoothed out the irregularities leaving the Universe isotropic and homogeneous, and with an entropy/baryon of $\sim 10^{40}$. When the temperature of the Universe dropped to about 10^{27} K ($kT \sim 10^{14}$ GeV) the baryon number and CP violating processes allowed the Universe to evolve a baryon/photon ratio of $\sim 10^{-9\pm1}$ erasing the only relic (baryon/entropy ratio $\sim 10^{-40}$) of a highly chaotic initial state.

One key assumption was tacitly made in the argument above: dissipation occurs before baryon generation. This assumption is needed because the baryon generation routes suggested thus far assume that the Universe is isotropic and homogeneous during the epoch of baryon creation ($kT \sim m_x$). In addition, any dissipation which occurs after the baryon excess is generated cannot be masked, as baryon number is very nearly conserved from that point forward. Thus we require dissipation to occur at a temperature $\gtrsim m_x/k$ ($\gtrsim 10^{27}$ K). It has been pointed out that dissipative processes during the quantum gravity epoch ($t \sim 10^{-43}$ s, $T \sim 10^{32}$ K) would have been highly efficient in dissipating chaos[5,6], so that it is reasonable to believe that the Universe was isotropic and homogeneous for temperatures $\lesssim 10^{32}$ K. Barrow is also considering the case when the dissipation does not precede the baryon generation (personal communication).

If the dissipation occurs early enough, a new, non-zero conserved quantity is needed to gain knowledge of the initial geometry. In some of the candidate theories for grand unification in addition to charge, some other number may be absolutely conserved, usually $B-L$ (B is baryon number, L is total lepton number). Suppose that the net charge of the Universe is zero, that the charged particles of the Universe are protons and electrons, and that the leptons of the Universe are electrons, and the neutrinos and anti-neutrinos in the 2 K neutrino background. Assuming further that the number of neutrinos and anti-neutrinos are exactly equal, the lepton number of the universe is equal to the number of electrons. Since the number of neutrons is about 1/7 the number of protons, the net $B-L$ number of the Universe is, within an order of magnitude, equal to the baryon number of the Universe. Then once again there is a conserved, non-zero quantity to compare to the entropy and it is possible to use the observed (net $B-L$)/photon ratio (of $\sim 10^{-9}$) to infer that the Universe began from a nearly isotropic and homogeneous initial state.

However, it is not easy to measure the lepton number of the Universe since the 2 K neutrino background is not detectable

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with present technology. Starting with the same assumptions as above except for the ratio of neutrinos to anti-neutrinos in the 2 K background which this time differs from 1 by $\sim +10^{-9}$: then for every $\sim 10^9$ photons there is one more neutrino than anti-neutrino. In this case the neutrino background contributes to the (net $B-L$)/photon $\sim -10^{-9}$, thereby reducing the (net $B-L$)/photon to nearly zero (say to 10^{-40}), whereas the visible (electrons, protons and neutrons) (net $B-L$)/photon is still $\sim 10^{-9}$. Therefore, while in principle the conserved quantity $B-L$ could be used to infer whether or not the initial geometry was isotropic and homogeneous, in practice, our inability to detect 2 K neutrinos makes this impossible.

The present day isotropy and homogeneity, and baryon/photon ratio of the Universe are important clues to the early history of the Universe. If a non-zero conserved quantity exists (such as baryon number), these two observations, as Penrose and others have pointed out, seem to imply the initial geometry of the Universe was nearly isotropic and homogeneous. However, if the grand unification ideas are correct and the baryon/photon ratio of $10^{-9\pm1}$ is generated in this way, then observed baryon/photon ratio, and the present isotropy and homogeneity do not allow us to infer that the initial geometry of the Universe was very nearly isotropic and homogeneous. In fact, the initial geometry could well have been highly chaotic.

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Quasar image doubling associated with absorption systems

Julian H. Krolik

Institute for Advanced Study, Princeton, New Jersey 08540

John Kwan

Bell Laboratories, Murray Hill, New Jersey 07974

Ever since their discovery the origin of quasar absorption lines has been a subject of considerable controversy (see ref. 1). In this note we consider the consequences of the hypothesis that at least some of the absorption lines arise in galaxies. These galaxies could be ordinary ones with 50–100 kpc haloes[2], or perhaps more compact but more common (at $z \sim 1$–2) objects of comparable mass.

In addition to selectively absorbing light of certain wavelengths, galaxies bend the path of the light by gravitational attraction. In the weak field approximation, the amount of bending in passing a point mass M at a distance l_P is $4M/l_P$ in units $G=c=1$ (ref. 3); if the mass is distributed and the light ray passes within it, the deflection, for the same total mass, can be slightly different depending on the details of the distribution[9,10]. The order of magnitude of the deflection angle in passing through a galaxy is $\sim 0.6''(\Delta v/250\ \mathrm{km\ s^{-1}})^2$, where $\Delta v = (GM/l_p)^{1/2}$ is the typical orbital speed in the galaxy. Such a small amount is invisible to ground-based optical telescopes (though not by a large factor), but should be easily discernible by either the Very Large Array (VLA) or the Space Telescope. We suggest, therefore, that those quasars with well defined absorption systems should be searched for gravitationally-focused multiple images.

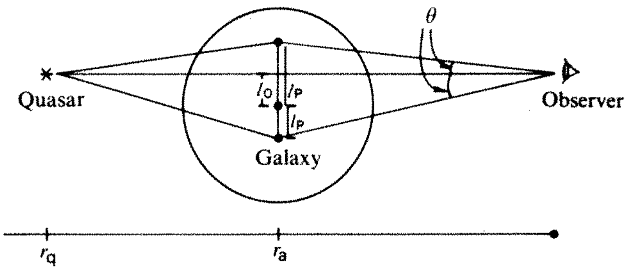

Fig. 1 The ray geometry is obviously not to scale. For precise definitions of the labels see text.

More precisely, we suppose that an absorption system of redshift z_a implies the presence of a galaxy at that redshift. We may find the angle by which the ray reaching Earth differs from the undeflected line of sight past the galaxy from the expression[4]:

$$4\left(\frac{\Delta v}{c}\right)^2 f(l_p)\frac{r_a r_{aq}}{(1+z_a)r_q} = l_p \mp l_0 = \frac{r_a\theta}{1+z_a} \tag{1}$$

where f is a slowly varying function of order unity whose form depends on the shape of the galaxy mass distribution; r_a and r_q are the comoving radial coordinates of the galaxy and the quasar, respectively, with respect to the observer as origin, whereas r_{aq} is the comoving radial coordinate of the galaxy with respect to the quasar as origin; l_0 is the proper distance from the centre of the galaxy to the undeflected line of sight and l_p is the proper pericentre distance of the ray; and $\theta (\ll 1)$ is the angle between the undeflected line of sight and the received ray (see Fig. 1). Here we have made the approximation that $l_p \simeq \pm l_0 + (r_a\theta)/(1+z_a)$; it is actually slightly less. The '±' correspond to paths that lie on the same, or opposite, sides of the galaxy's centre as the undeflected line of sight. We have also assumed that there is no other scattering along the ray path. In cosmologies which are homogeneous only in the mean so that the rays propagate in empty space but are subjected to many weak scatterings[5,6], the results differ by <10%.

The function $f(l_p)$ accounts for the galaxy's mass distribution. If it is spherically symmetric and has a flat velocity curve from inside l_p out to a radius l_1 (but has no mass outside l_1), then we have (for $l_p < l_1$):

$$f(l_p) = \tfrac{1}{2}\left(\cos^{-1}(l_p/l_1) + 2(l_1/l_p)\{1-[1-(l_p/l_1)^2]^{1/2}\}\right)$$
$$\simeq \begin{cases} \tfrac{1}{4}[\pi + \tfrac{1}{6}(l_p/l_1)^3] & l_p/l_1 \ll 1 \\ 1 & (l_1-l_p)l_1 \ll 1 \end{cases} \tag{2}$$

If $l_p \geqslant l_1$, $f(l_p) = l_1/l_p$.

The easiest way to find the actual splitting angle (given l_0, Δv, and the cosmological information) is to use the graphical construction of Fig. 2. The splitting angle is:

$$\theta_{\mathrm{sep}} = 4[f(l_{p+}) + f(l_{p-})]\left(\frac{\Delta v}{c}\right)^2 \frac{r_{aq}}{r_q}$$
$$\simeq 0.9'' r_{aq}/r_q \left(\frac{\Delta v}{250\ \mathrm{km\ s^{-1}}}\right)^2 \tag{3}$$

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where $l_{p\pm}$ are given by the two intersections of the straight lines with the curve in Fig. 2.

Clearly, there are only solutions for both l_{p+} and l_{p-} if:

$$l_0 \leqslant \pi\left(\frac{\Delta v}{c}\right)^2 \frac{r_a r_{aq}}{(1+z_a)r_q} = 13\,\frac{r_a r_{aq} H_0}{(1+z_a)r_q c}\left(\frac{\Delta v}{250\text{ km s}^{-1}}\right)^2 \times\left(\frac{H_0}{50\text{ km s}^{-1}\text{ Mpc}^{-1}}\right)^{-1}\text{kpc} \qquad (4)$$

To evaluate this limit, we note that[3]:

$$r_a = \frac{c}{H_0(1+z_a)q_0^2}\{z_a q_0 + (q_0-1)[-1+(1+2q_0 z_a)^{1/2}]\} \qquad (5)$$

with an analogous expression obtaining for r_q, while

$$r_{aq} = \frac{c}{H_0 q_0^2(1+z_a)(1+z_q)}[(1-q_0+q_0 z_q)(1+2q_0 z_a)^{1/2} - (1-q_0+q_0 z_a)(1+2q_0 z_q)^{1/2}] \qquad (6)$$

where z_q is the quasar redshift and q_0, H_0 are evaluated at the present epoch. For 'typical' numbers ($q_0 \leqslant 0.1$, $0.5 \leqslant z_a$, $z_q \leqslant 3.5$), the maximum undeflected impact parameter that will produce a double image is only

$$\sim 2\left(\frac{\Delta v}{250\text{ km s}^{-1}}\right)^2\left(\frac{H_0}{50\text{ km s}^{-1}\text{ Mpc}^{-1}}\right)^{-1}\text{kpc}$$

(see Table 1).

Consequently, only a small fraction, $\langle (l_{0,\max}/l_1)^2\rangle$, of all absorption systems will produce double images. To produce the six or so absorption systems which are commonly found in quasars of $z_q \gtrsim 2$,

$$l_1 \simeq 100\text{ kpc}\left(\frac{H_0}{50\text{ km s}^{-1}\text{ Mpc}^{-1}}\right)^{-1}$$

(ref. 7). This estimate of l_1 assumes the present number density of galaxies and no galaxy–quasar correlations; it would decrease if the density of objects gravitationally bound as tightly as galaxies and at least a few kiloparsecs in size were greater at $z \sim 1$–3, or if correlations do exist. Using $l_1 \simeq 100$ kpc, we estimate the probability per absorption system that a double image is formed to be $O(10^{-3})$. Unfortunately, the close approach of the rays to the galaxy centre also entails the hazard that visible wavelengths may be extinguished by dust, or that the surface brightness of the galaxy may mask the quasar images.

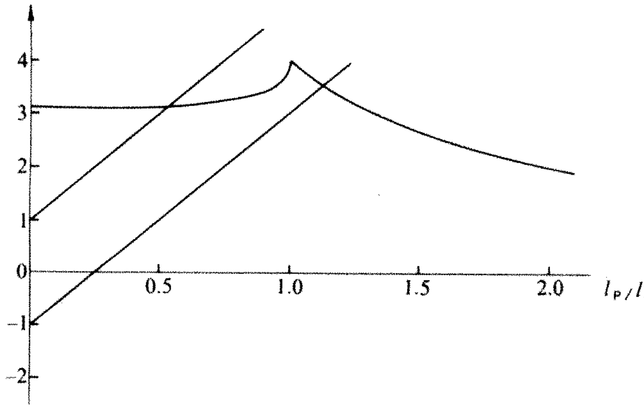

Fig. 2 The curve shows $4f(l_p)$ as a function of l_p/l_1 for the mass model that led to equation (2). The lines represent $(l_p \pm l_0)\cdot(1+z_a)r_q/[r_a r_{aq}(\Delta v/c)^2]$ as a function of l_p/l_1, where we have chosen the illustrative parameters:

$$\frac{r_a r_{aq}}{(1+z_a)r_q}\left(\frac{\Delta v}{250\text{ km s}^{-1}}\right)^2\left(\frac{l_1}{100\text{ kpc}}\right)^{-1}\left(\frac{H_0}{50\text{ km s}^{-1}\text{Mpc}^{-1}}\right)^{-1} = 6$$

and $l_0/l_1 = 0.25$.
Their intersections with the curve give the approximate pericentres of the received rays: $l_p/l_1 \simeq 0.53$ and 1.13. As l_0/l_1 increases, the intercepts of the straight lines with the vertical axis spread until eventually only the ray corresponding to the − sign is received.

Table 1 Sample values of θ_{sep} and the maximum ray impact parameter $l_{0,\max}$

z_a	r_a	r_{aq}	$l_{0,\max}$(kpc)	θ_{sep}(arc s)
0.25	0.22	1.04	1.76	0.69
0.5	0.41	0.81	2.10	0.54
1.0	0.71	0.50	1.70	0.33
1.5	0.95	0.29	1.06	0.19
2.0	1.17	0.13	0.49	0.086

$q_0 = 0.1$ and $z_q = 2.5(r_q = 1.364c/H_0)$
The values of r_a and r_{aq} are in units of c/H_0; $l_{0,\max}$ and θ_{sep} are calculated assuming $(\Delta v/250\text{ km s}^{-1})^2 \times (H_0/50\text{ km s}^{-1}\text{Mpc}^{-1})^{-1} = 1$.

Another way of understanding this probability estimate is to use the result[5] that if the mass of the Universe comes in many point-like objects, the probability of creating a visible double image of an object at a cosmological distance is of order Ω, the density parameter. Here, in effect, only the mass contained in the inner few kiloparsecs contributes to Ω.

There are two types of checks that may be made on a double image, to confirm that it is due to gravitational deflection. The first, which must await the Space Telescope, is to match the spectra of the two images. In general, radio spectra are too smooth to attach much significance to matching their shapes. The second is to match the images' time-variability records. The difference in path lengths will be

$$\leqslant 2\left(\frac{\Delta v}{250\text{ km s}^{-1}\text{Mpc}^{-1}}\right)^4\left(\frac{H_0}{50\text{ km s}^{-1}\text{Mpc}^{-1}}\right)^{-1}(r_{aq}/r_q)^2\text{ months,}$$

depending on how nearly symmetrical the ray paths are about the galaxy centre. Even if the two images are not resolved, the quasar can be examined for doubling by performing an autocorrelation in time of its intensity (S. Tremaine, personal communication).

The autocorrelation function will always have its strongest peak at zero delay, but if the intensities of the images are not too different, a second peak should appear that corresponds with the difference in path lengths. To estimate that intensity ratio, we use the fact that the gravitational lens conserves surface brightness. Then each image's intensity is multiplied by a factor $l_p/l_0|dl_p/dl_0|$ (ref. 8); in our case, $d\ln f(l_p)/d\ln l_0 \ll 1$, so the factor reduces to $\simeq l_p/l_0$. Thus we expect an intensity ratio of perhaps a factor of two or so, and the autocorrelation test should be feasible.

The plausible idea that at least some quasar absorption systems are made in galaxies implies that a small fraction of these galaxies will cause a doubling of the quasar image by gravitational focusing. The separation of these images ($\leqslant 1''$) should be detectable with the VLA (for radio quasars) and the Space Telescope when it flies. Autocorrelation of the variability of unresolved quasar images may also reveal doubling due to gravitational focusing.

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Scaling law test and two predictions of planetary magnetic moments

C. T. Russell

Institute of Geophysics and Planetary Physics, University of California, Los Angeles, California 90024

The scaling law for the strength of planetary magnetic dynamos, originally derived from a magnetohydrodynamic (MHD) theory[1], has recently been independently re-derived using simple dimensional analysis[2]. While dimensional analysis is certainly no replacement for a solution based on a physical mechanism, this analysis does lend more credence to the scaling law by the implicit suggestion that the form of the scaling law is not an artefact of the MHD solution. We show here that the scaling law holds quite well in ordering the three well determined planetary magnetic moments. The present meagre data suggest that the terrestrial magnetic moment is about twice the value expected when compared with the other planets. Assuming that Earth is anomalous, rather than Mercury and Jupiter, and that the scaling law is valid, we predict that Saturn will be found to have a moment of 1.2×10^{29} G cm^3 and Io a moment of 6.5×10^{22} G cm^3.

Expressed in terms of magnetic moments which are the observable parameters in planetary exploration, the scaling law becomes

$$\frac{M_P}{M_E} = \left(\frac{\rho_P}{\rho_E}\right)^{1/2} \left(\frac{R_P^c}{R_E^c}\right)^4 \frac{\Omega_P}{\Omega_E}$$

where M is the magnetic moment, R^c is the radius of the core, ρ is the density of the core and Ω is the spin rate. The subscript P refers to the planet, E to the Earth. For this scaling law to work, all planetary dynamos must have the same underlying physical mechanism. We note also that the strength of the energy source for the dynamo does not appear in this scaling law. In this model if the strength of the energy source is not sufficient, the dynamo does not regenerate and then stops. If it is regenerative, it assumes a particular strength given by the scaling law. This lack of consideration of the strength of the energy source is considered by many to be grounds for rejection of any scaling law.

In the scaling law Ω is well known for all large bodies in the Solar System except for the outermost planets. However, there is much uncertainty in the core radius and density, especially for the inner planets whose chemical composition and thermal history are uncertain. Nevertheless, enough modelling has been done, and planetary measurements performed for us to be able to put this scaling law to a serious test.

The results of this test are shown in Fig. 1. For Mercury we have made the core density the same as that of the Earth and the limits on core radius to be 1,470–1,840 km (refs 3, 4). The range of the observed magnetic moment was taken to be from 2.4×10^{22} to 5×10^{22} cm^3 (refs 5–7). For Venus we have taken a core density equal to that of the Earth and a fractional radius within 10% of that of the Earth. The magnetic moment has been taken to be less than 1×10^{22} G cm^3 (ref. 8). For the Earth we have taken a moment of 8×10^{25} G cm^3, a radius of 3,486 km, and a density of 11 g cm^{-3} (ref. 9). For Mars, we have taken a range of models with realistic core densities given by Okal and Anderson[10] for the internal structure and the magnetic moment of Dolginov and coworkers[11] as an upper limit[12]. For Jupiter we use the models of Stevenson and Salpeter and its stated uncertainties[13] and the moment of Davis and Smith[14].

The straight line through the Earth has a slope of unity. We would expect that those planets with operating dynamos would fall on this line. Clearly Jupiter and Mercury do not. However, they both fall on a slightly displaced line with unit slope. Thus relative to Jupiter and Mercury the Earth seems to be anomalously high. It might be said that since the three planets with clearly established dynamos do not fall on one line, then the scaling law has failed. However, for the sake of further discussion, we will assume that the scaling law works but that the Earth is anomalous.

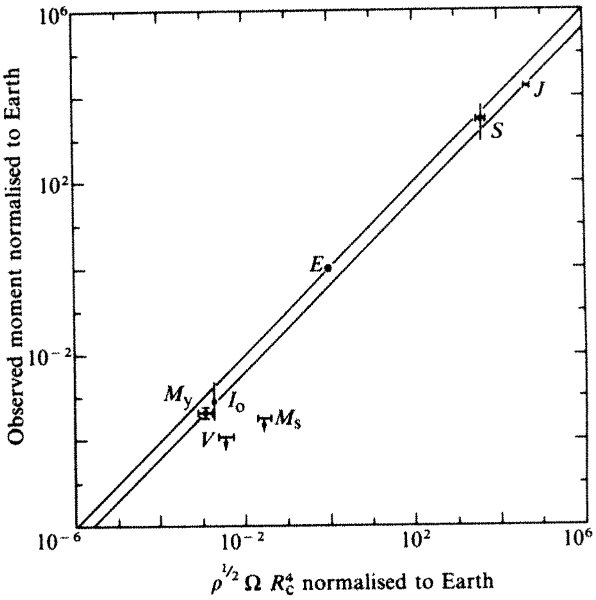

Fig. 1 Application of the scaling law to Earth (E); Mars (M_s); venus (V); Mercury (M_y); Saturn (S); Jupiter (J) and Io.

Venus and Mars do not lie near either line which suggests that they do not have dynamos at present. If one were to detect unambiguously an intrinsic field at Venus or Mars due to an internal dynamo at or below these limits, this observation would necessitate reassessment of Busse's dynamo theory.

For Saturn we have taken Slattery's model[15] and arbitrarily assumed ±6% for the uncertainty in core radius. The dot marks the moment inferred from radio observations[16]. This data point falls squarely between the jovian and terrestrial scaling lines. If we assume that the jovian scaling line is the proper scaling line with which to compare Saturn we would predict a core radius for Saturn of 33,000 km, much greater than the 28,000 km assumed by Slattery. If the modelling is correct, then the expected moment is 1.2×10^{29} G cm^3 corresponding to an equatorial surface field of 0.5 G. While it is difficult to judge which is the more accurate, the theoretical modelling of Saturn's interior or the scaling of the radioemissions, we argue that as the source of the radioemissions is not understood for the Earth, Jupiter or Saturn, the modelling effort is more accurate at present. Thus, our first prediction is that Pioneer 11 will measure a moment of about 1.2×10^{29} G cm^3. If the jovian modelling efforts can be of any guide we expect this estimate to be correct to about ±20%.

Finally let us examine Io. A prediction for Io has already been made by Neubauer[17]. He assumes an Fe–FeS eutectic core with a density of 5.3 g cm^{-3} and a core radius of 890 km corresponding to an average chondritic composition. Voyager pictures of Io show many active volcanoes suggesting that Io is molten internally. This in turn suggests Io has in fact differentiated and formed an iron core. Thus far the Voyager mission has not provided further constraints on the size of the core. However, it is clear that the satellite is losing sulphur. Thus we will also use a higher core density, 7 gm cm^{-3} (ref. 10) in our estimates of the expected moment. This expectation is indicated by the vertical line which crosses the Earth scaling law at a moment of 1.3×10^{23} G cm^3, very close to Neubauer's prediction. The line crosses the Jupiter–Mercury line at 6.5×10^{22} G cm^3. This same moment has been inferred from observations of Io's ionosphere and effects on particle motions by Kivelson and coworkers[18]. Our second prediction, based on the adoption of the Jupiter–Mercury line as the best scaling line, is that Io has a moment of

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close to 6.5×10^{22} G cm^3. The weak link in this prediction is the choice for the size of the core of Io.

If the above predictions are correct, we must seek an explanation for why the Earth's field is anomalously high, as the scaling law does not involve the strength of the energy source. Perhaps the answer lies in the precessional torques applied to the Earth's core by the Moon.

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Note added in proof: On 1 September 1979, Pioneer 11 flew by Saturn and detected a magnetic moment of 4.8×10^{28} G cm^3 (E. J. Smith, personal communication). To be consistent with the Busse scaling law, the conducting core of Saturn would have to be 23,000 km in radius. Unless future analysis of the Pioneer 11 gravity data and a reassessment of the models of the interior structure are consistent with such a small core we must conclude that Busse's scaling law is useful for predicting planetary moments only to within a factor of about 2.5.

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Isotope effects and correlated motion in self and sodium ion diffusion in water

L. W. Barr & M. A. M. I. Elmessiery

Paisley College of Technology, Paisley, Scotland, UK

The information gained on atomic motions in solids from studies of the isotope effect in diffusion[1,2] prompted us to examine this effect in liquids, in particular for diffusion in water, because of its importance as a solvent. As isotope effect measurements for self-diffusion in water and deuterium oxide are available[3], the isotope effect for ^{22}Na and ^{24}Na ions in water was studied. We describe here a new technique which applies to liquids the solid state technique of simultaneous diffusion[4] of these isotopes followed by sectioning and half-life (or other suitable method) separation.

A dilute sodium chloride solution (0.034 mol l^{-1}) was gelled by the addition of between 1 and 3% agar (Ion Agar No. 2) and cast into a tube from which it could be pushed by an accurate screw thread and plunger for sectioning into slices 1–3 mm thick. Suitable quantities of ^{22}NaCl (carrier free ^{22}Na, Radiochemical Centre, Amersham) and ^{24}NaCl (prepared by irradiation of NaCl in the Scottish Universities Research Reactor) in aqueous solution were dripped onto a freshly cut gel surface from a hypodermic syringe before the tube was capped for annealing in a temperature controlled bath.

After sectioning, the gel slices were weighed and centrifuged down into suitable counting tubes to provide a reproducible counting geometry and each counted repeatedly in a well-type NaI scintillation counter until the 15-h half life ^{24}Na had completely decayed. From the decay curve for each section the specific activities C_{22} and C_{24} for each tracer could be calculated.

Plots of the log of the specific activity against the square of the penetration distance were accurately linear over a two decade range in activity, as required by the appropriate solution of Fick's equation. Moreover, comparison of the resulting diffusion coefficients with those obtained by conventional methods showed that the sodium diffusion was taking place through the water phase.

The isotope effect was then calculated from the equation[16]

$$\ln (C_{22}/C_{24}) = \text{constant} - (D_{22}/D_{24} - 1) \ln (C_{22})$$

The results obtained for the isotope effect for sodium diffusion in water are listed in Table 1 together with the self-diffusion results of Mills[3] and one value for ^{6}Li and ^{7}Li calculated from the limiting conductance data of Kunze and Fuoss[5].

The results were analysed using Schoen's equation[6]

$$\left(\frac{D_\alpha}{D_\beta} - 1\right) = f \, \Delta K \left[\left(\frac{m_\beta + nm_0}{m_\alpha + nm_0}\right)^{1/2} - 1\right] \qquad (1)$$

where D_α/D_β is the ratio of the diffusion coefficients of the two isotopes in the same solvent, f is the correlation factor for the diffusion process, m_α and m_β are the masses of the isotopic tracers and n and m_0 the number and average mass of any non-tracer molecules involved in the diffusion movement. In solids ΔK is the fraction, possessed by the diffusing species, of the total translational kinetic energy associated with the decomposition of the saddle point configuration[1]. Clearly $0 \leq \Delta K \leq 1$ and in many metals, particularly the close packed metals[2], ΔK is close to unity.

Although Schoen's equation is exact only in certain conditions[1,7], it has been applied to glass[8] and benzene[9] where the required symmetry conditions would not be met. Water has a structure which, at least over short distances, approximates to a tetrahedral arrangement of water molecules[10–13] with Z, the coordination number, lying between 4.4 and 5.5. For such a molecular arrangement equation (1) should hold at least to the precision justified by the experimental data.

Table 1 shows values of $f\Delta K$ calculated from equation (1). For water $n = 0$, while for sodium and lithium m_0 is the mass of a water molecule and a value of $n = 4.5$ was used in view of the known hydration of alkali ions in water. If the hydration number is defined as the number of water molecules which have surrendered their own translational freedom and remain with the ion when it moves relative to the surrounding water, then recent reviews[14,15] suggest that the most probable integral value for n is 4 ± 1 for sodium and 5 ± 1 for lithium. The value of $n = 4.5$ chosen is then a very convenient test value for both ions: it is probably close to the best possible estimate for sodium[15] but may be slightly low for lithium.

It is encouraging that the values of $f\Delta K$ so calculated for water and the hydrated alkali ions are so similar. This is particularly true for the alkali ions since in that case the experimental techniques used were so different.

It has been shown elsewhere [1,16] that the correlation factor f_0 for self-diffusion by a vacancy or hole mechanism can be approximated with a fair degree of accuracy by

$$f_0 = 1 - \frac{2}{Z} \qquad (2)$$

where Z is the coordination number. This is very important when applying the idea of correlated motion to liquids since long-range order can be neglected and attention focussed on the number of neighbours in the first coordination shell.

The striking agreement between the experimental $f\Delta K$ for water and the range of values of f_0 calculated from equation (2) supports a hole mechanism for diffusion in water and suggests that ΔK is close to unity, $f_0 \simeq 0.62$ and Z is about 5 (ref. 12). Note that a ΔK of 1 implies that the frequency with which a tracer moves into a hole is inversely proportional to the square root of its mass and this is in line with theoretical predictions[17].

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Note that molecular motion in water may be strongly correlated. Indeed, as

$$D\ (\text{measured}) = D(\text{random})\, f_0$$

the observed diffusion coefficient may be about 40% less than would be calculated on the basis of random motion. This accords with predictions made on the basis of molecular dynamics[18]. This interpretation differs from that of Dunlop[19] who suggested that $f_0 = 1$ and $\Delta K \simeq 0.5$.

For sodium and lithium also ΔK can be assumed to be close to one. For sodium f would be expected to exceed that for water diffusion as it is a slow diffusing impurity.

The results shown in Table 1 can be interpreted on the basis of a crude model. Suppose each alkali ion is surrounded symmetrically by between 4 and 6 hydrated water molecules[20], the whole loosely contained in a cage of free water. On average the hydrated water will have fewer free molecules adjacent to it than a free molecule because of the presence of the sodium ion and the remainder of its hydration cell on one side. If a complete hemisphere were forbidden then each hydrated water molecule would have on average 2.5 free molecules adjacent to it. If we assume an integer value of 3 for the average then each hydrated water with its three free water molecules and the alkali ion forms a structure having a coordination number of four.

Suppose that a space occurs in the shell of free water adjacent to a hydrated water molecule. That hydrated molecule can then move into the space carrying with it the hydrated ion. This produces a space next to the free molecules on the opposite side of the hydrated ion. One of these free molecules could move to occupy the space or alternatively the hydrated alkali ion could move back to occupy its original position.

This motion of hydrated alkali ions and free molecules closely resembles an impurity molecule diffusing on a diamond lattice for which an expression for f is available[1]. If the relative probabilities of a hydrated or free water molecule moving into a space are described in terms of jump frequencies ω and ω_0 then the correlation factor for hydrated alkali ion motion in such a structure is

$$f = \frac{\omega_0}{\omega + \omega_0} \tag{3}$$

which for the case of self-diffusion where $\omega = \omega_0$, reduces to $f = 0.5$ as required by equation (2). If $\omega_0 > \omega$ then $f > f_0$.

If we assume that the probability of a hole forming in the shell adjacent to a hydrated ion is the same as in pure water then, as the mean distance moved in each step is the same for free or hydrated water, the ratio of the coefficients of self and impurity diffusion can be written as

$$\frac{D(H_2O)}{D(\text{ion})} = \frac{\omega_0 f_0}{\omega f} \tag{4}$$

This assumption is plausible as both sodium and lithium are 'structure-forming' ions, known not to perturb the structure of water. In the case of lithium, molecular dynamical calculations also suggest that the Li^+ ion causes relatively little disturbance of the structure of the surrounding water[20]. Note that this assumption cannot be extended to other ions. For example, the addition of caesium ions to water enhances water self-diffusion[21], probably because of a greater ease of formation of holes adjacent to the caesium ion.

Table 1 Isotope effects and correlation factors for self and impurity diffusion in water

System	Temperature (°C)	$(D_\alpha/D_\beta - 1)$	$f\Delta K$	f_0 (Theory)
HDO/HTO	1	0.0135	0.52	
in	5	0.0181	0.69	0.55
H_2O	25	0.0161	0.62	to
	45	0.0167	0.64	0.64
	Average	0.0161	0.62	
HDO/DTO	5	0.0330	0.64	
in	15	0.0287	0.56	0.55
D_2O	25	0.0299	0.58	to
	Average	0.0305	0.59	0.64
$^{22}Na/^{24}Na$	19	0.0059		
in	19	0.0065		
H_2O	19	0.0056		
	30	0.0066	0.63 ± 0.03	
	39	0.0057		
	50	0.0062		
	Average	0.0061		
$^6Li/^7Li$ in H_2O	25	0.0035	0.61	

Table 2 Correlation factors and hydration numbers for alkali diffusion in water at 25°C

System	Observed ratio	Calculated ratio	f	n	ω_0/ω
$\frac{D(H_2O)}{D(Na)}$	$\frac{2.236}{1.296} = 1.72$	1.68	0.64	4.6	1.8
$\frac{D(H_2O)}{D(Li)}$	$\frac{2.236}{1.030} = 2.17$	1.59	0.71	5.3	2.5

The units of D are $10^{-5}\ cm^2\ s^{-1}$. The values of the tracer diffusion coefficients of water and sodium are from refs 3 and 22. The value for lithium was calculated from the limiting conductance, ref. 23.

Eliminating ω_0/ω from equations (3) and (4) yields the simple relationships

$$\frac{D(H_2O)}{D(\text{ion})} = \frac{f_0}{1-f} \tag{5}$$

and

$$\frac{\omega_0}{\omega} = \frac{f}{1-f} \tag{6}$$

for this two frequency model.

Table 2 gives experimental values for the left-hand side of equation (5) at 25°C for sodium and lithium together with calculated values using the f and f_0 values from Table 1. The agreement is surprisingly good, particularly for sodium, considering the crudeness of the model and the assumptions made.

An alternative application of equation (5) is to use the experimental values of $D(H_2O)/D(\text{ion})$ and f_0 to calculate f and then, using the experimental values for the isotope effect and Schoen's equation, (1), to calculate independently values of the hydration number, The resulting values of f, and n are shown in Table 2.

The values of n so obtained are in excellent agreement with those obtained by other methods and support the conclusion that the hydration number for lithium slightly exceeds that for sodium.

Finally, values of ω_0/ω calculated using the f values from Table 2 are also listed. In the case of lithium this ratio is almost equal to the inverse square root of the masses of the hydrated ion and water.

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Solar bacterial biomass bypasses efficiency limits of photosynthesis

Helmut Tributsch*

Fritz-Haber-Institut der Max-Planck-Gesellschaft, D-1000 Berlin 33, Faradayweg 4-6, FRG

A new kind of solar energy technology is proposed here which will allow biomass to be produced at efficiencies higher than the theoretcial limits of photosynthesis and at one order of magnitude higher than by present processes. Its principle is to produce the chemically simple inorganic energy source of certain autotrophic bacteria by solar thermal, photoelectrochemical or solar powered electrochemical methods and to grow these bacteria, for example *Thiobacillus ferrooxidans*, which can live from the oxidation of ferrous to ferric iron in continuous cultures in the dark. A remarkable prospect of this novel strategy for gathering solar energy would be that biomass for food, fuels and materials could be produced in largely automatic and compact factory-type installations on arid and infertile land, to which the limited amount of water which is needed to fix CO_2 and is extracted in the form of dry organic products may be transported as a raw material.

The efficiency limits of photosynthesis are well known. Insufficient photon energy and incomplete utilisation of photon energy reduce the upper theoretical limits of the conversion efficiency to approximately 11% of the total solar radiation on Earth. Inevitable nongenerative losses and plant system losses (such as reflection, and metabolic conversions) further reduce the maximum practical conversion efficiency to 5–6%[1]. High daily yields approaching this maximum have been reported, but even very productive tropical grasses such as sugar cane do not yield crops at efficiencies of more than 0.6% on an annual basis[2]. The water hyacinth, which is considered to be one of the most efficient solar energy converters is expected to be capable of yields of 45–60 tonnes acre^{-1}, corresponding to ~3–4% efficiency under careful management[1]. Unfortunately, the need for fertile soil and abundant sweet water restricts biomass production mostly to regions which to a large extent are already cultivated. It cannot be extended to the huge unused desert regions of the world where solar energy is at its most intense.

The rather low efficiency limits of biomass production and the high loss of water which is primarily due to evaporation from the plants and the soil can be overcome by the proposed biomass technology. This could replace the inefficient multi-photon biological mechanism of conversion of solar energy into energy-rich primary products by more efficient artificial solar techniques, which could provide either concentrated solar heat or solar electricity for the production of chemically simple inorganic energy carriers on which microorganisms feed.

I have cultivated and studied the bacterium *T. ferrooxidans* for several years[3], and to demonstrate the feasibility of such a solar technology I have theoretically investigated a biomass installation, in which this bacterium is grown on ferrous iron produced from ferric iron by a solar thermal method. In analogy to the generation of hydrogen and oxygen from water by thermal processes many chemical cycles can be proposed which evolve oxygen from water and reduce ferric ions instead of protons. The estimated efficiencies of proposed thermal processes to generate H_2 and O_2 from water range from 30 to 50% (refs 4–7) and will not be lower for the production of ferrous iron, which has a considerably more positive redox potential than that of hydrogen. While no practicable thermochemical processes with less than three steps are known for the production of H_2 and O_2 from water, two-step processes are possible for the generation of ferrous iron and oxygen. An example are the reactions:

$$\begin{aligned} 2\,\mathrm{FeCl_3} &\xrightarrow{350\,^{\circ}\mathrm{C}} 2\,\mathrm{FeCl_2} + \mathrm{Cl_2} \\ \mathrm{H_2O} + \mathrm{Cl_2} &\xrightarrow{700\,^{\circ}\mathrm{C}} 2\,\mathrm{HCl} + \tfrac{1}{2}\mathrm{O_2} \end{aligned} \tag{1}$$

T. ferrooxidans requires an acid environment ($pH = 1.2$–4.5) and obtains its energy for growth from oxidising ferrous iron, metal sulphides, sulphur or soluble inorganic sulphur compounds. It grows in strictly autotrophic conditions (optimum at 35 °C), obtaining all the carbon required for biosynthesis from atmospheric carbon dioxide which it fixes by biochemical mechanisms that are part of or related to the Calvin cycle[8]. Ammonium is a suitable source of nitrogen, but evidence for the ability of *T. ferrooxidans* to fix atmospheric N_2 has been presented[9]. The bacterium develops a very high tolerance for metal ions. Cl^- retards Fe^{2+} oxidation at concentrations above $6\,\mathrm{g\,l^{-1}}$, but tolerance to cultivation in hydrochloric acid can be developed and its growth on iron in a medium where all iron is supplied as the chloride is possible provided some additional sulphate is available[10]. *Thiobacillus ferrooxidans* can therefore be cultivated on $FeCl_2$ supplied by the solar thermal process (1) and thereby recycles $FeCl_3$ and water:

$$2\,\mathrm{FeCl_2} + 2\,\mathrm{HCl} + \tfrac{1}{2}\mathrm{O_2} \longrightarrow 2\,\mathrm{FeCl_3} + \mathrm{H_2O} \tag{2}$$

T. ferrooxidans accomplishes the oxidation of Fe^{2+} at rates 10^5–10^6 times faster than the rates of inorganic oxidation at the corresponding pH (ref. 8). The energy which it thereby gains depends on the pH and ranges from $7.84\,\mathrm{kcal\,mol^{-1}}$ (pH 1.5) to $5.89\,\mathrm{kcal\,mol^{-1}}$ (pH 3). For the reduction of CO_2 to the level of carbohydrate approximately $118\,\mathrm{kcal\,mol^{-1}\,CO_2}$ are needed. For an output of $7\,\mathrm{kcal\,mol^{-1}\,Fe^{2+}}$ about $16\,\mathrm{g\,mol^{-1}\,Fe^{2+}}$ would be consumed to fix $1\,\mathrm{g\,mol^{-1}\,CO_2}$. Per gram mol Fe^{2+} (56 g) a maximal theoretical cell yield of approximately 1.4 g dry weight could therefore be expected[8]. The energy conversion efficiencies actually obtained by *T. ferrooxidans* grown on ferrous iron have steadily improved with the development of improved strains and the use of continuous flow chemostat cultures. A detailed recent analysis of growth yields and total energy balance at pH 1.6 is given by Kelly *et al.*[11], which suggests that $19\,Fe^{2+}$ ions are oxidised per CO_2 molecule fixed. The true growth yield Y_G (g dry weight per atom Fe^{2+}, if no substrate were used for the maintenance of the bacteria) was found to be 1.33, corresponding to an energy conversion efficiency of 78.8%. The yield actually obtained, which includes the energy losses due to cellular processes and is subject to non-competitive and competitive product inhibition by the ferric iron, varied depending on the condition of cultivation between 0.156 and 0.8 g dry weight per g atom Fe^{2+} (ref. 11). A dry weight yield of 0.5 g per g atom Fe^{2+} corresponding to an efficiency of 35% on a free energy basis has also been reported[8,12] and I believe that is a reasonably achievable value for large scale technical biomass production by *T. ferrooxidans* in continuous culture.

If this efficiency ($\eta_2 = 35\%$) is combined with the efficiency for the solar thermal generation of ferrous iron ($\eta = 30$–50%) a total solar energy efficiency for biomass production of $\eta =$

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[*] Present address: CNRS, Laboratatoire de Bellevue, 92190 Meudon, Paris, France.

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$\eta_1\eta_2 = 10.5\text{–}17.5\%$ is obtained for the proposed solar technology. On an annual basis it would thus be 17.5 to nearly 30 times higher than the biomass yield from a sugar cane field of comparable area. The bacterial biomass would be very protein-rich (*T. ferrooxidans* contains 44% protein, 26% lipid, 15% carbohydrate and at least two B-vitamins[13]), has no known negative physiological effect and could be collected in installations of moderate size, since *T. ferrooxidans* can be cultivated on high concentrations of ferrous iron, achieving growth rates exceeding 0.2 h^{-1} and doubling times <3.5 h in continuous flow cultures[14]. Using published experimental data[11] biomass yields of 70 kg dry wt m^{-3} yr^{-1} can already be estimated for continuous cultures in their present stage of development. Single cell protein factories have already been shown to be feasible in the case of petroleum conversion, and problems arising from the processing of the material for specialised use seem to be solvable.

What would a solar bacterial biomass (SBB) plant look like? Built as a solar tower installation with a collector field of 1 km radius in a desert it would cover an area of 3.14 km^2. As not all the incident sunlight could be collected in the central receiver the input of solar energy would approximately correspond to that of a comparable sugar cane field in a tropical region with inferior insulation. However, up to 30 times more biomass could theoretically be extracted from the SBB installation which could be built on infertile soil. The thermochemical reactions (1) and the energy supplying reaction (2) on which bacterial biomass is grown recycle the water needed to cultivate *T. ferrooxidans* and the only real loss of water is due to the biological fixation of CO_2. If biomass is extracted from the SBB plant as dry material the weight equivalent of water which would have to be replaced would be smaller or—if some losses are taken into account—of a similar order of magnitude. Provided that the water in the SBB installation is periodically cleaned, which could occur during the thermochemical step (1), all that may be needed is that those transportation vehicles which carry away dry biomass also supply water to the plant. The total amount of water needed to operate a SBB plant would fill tanks (10 m high) which cover <10% of the collector area (mirrors can be placed on top of these). The addition of a fertiliser to the culture medium will be necessary but the bacteria's apparent ability to utilise nitrogen from the air[9] could be developed. (A standard culture medium has been described in ref. 15).

The described energy collecting cycle for a SBB installation based on *T. ferrooxidans* and a solar thermal process is just one technical possibility which is especially interesting since it promises high theoretical efficiencies. One disadvantage, which is common to all thermochemical cycles, is that relatively large quantities of material (Fe^{2+}, Fe^{3+}) would have to be circulated to maintain a relatively small flow of energy. The simplicity of the reactions involved (1)(2), and a skilful technical design (for example, utilisation of thermal convection) might keep such additional energy losses within reasonable limits. They would not occur if the solar thermal process for the generation of Fe^{2+} were replaced by a solar powered electrochemical one (current generation by solar cells, ocean thermal gradient, wind, wave power) or a photoelectrochemical one in which ferrous iron is produced in the cathodic reaction and oxygen in the anodic reaction of an electrochemical cell. In this case the bacteria could in principle be grown in the same reactor in which their energy source is produced and they would be converting irregularly supplied electrical energy into valuable organic products. The total efficiency ($\eta = \eta_1\eta_2$) which would seem to be feasible in this case is of the order of 6%. The same bacterium *T. ferrooxidans* could also be cultivated on sulphur, thiosulphate, many metal sulphides or a combination of metal sulphides with ferric iron provided the energy supplying inorganic substances can be efficiently recycled from the oxidation product sulphate by a solar thermal or solar electrochemical process. *Thiobacillus thiooxidans* could be grown on sulphur or inorganic sulphur compounds and *Knallgasbacteria* on solar hydrogen. There are other bacterial species which feed on energy sources which are chemically sufficiently simple to be synthesised by solar technological methods.

The suggested SBB technology is based on established principles and does not involve expensive raw materials. It also seems that certain practical problems which will arise (for example, the separation of bacterial biomass from ferric iron) could be handled with appropriate applied research. These preconditions, the promise of biomass yields, which are one order of magnitude higher than that from present day agriculture, extreme economy of water utilisation and the prospect of compact and largely automatic installation make this a practical proposition. However, SBB installations will only become commercially interesting after large-scale solar plants have proved to be feasible. A SBB technology would seem to be rewarding since the combination of modern solar technology with inorganic bacterial oxidation would break down some of the restricting environmental conditions imposed on the solar synthesis of biological products and enlarge the surface area of the world suitable for production of food, energy and materials.

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Amino acid ratios and the correlation of raised beach deposits in south-west England and Wales

J. T. Andrews

Department of Geological Sciences and INSTAAR, University of Colorado, Boulder, Colorado 80309

D. Q. Bowen & C. Kidson

Department of Geography, University College of Wales, Aberystwyth, UK

Interest in studies of amino acid racemisation as a framework for Quaternary chronostratigraphy has increased recently[1–4]. These studies have used different materials, but the main two substances have been bone and shell. Different shell genera can have different rates of racemisation[5], so the use of new genera requires some study of the kinetics of the reactions before the results from one taxa can be compared to another. We report here the successful application of amino acid racemisation studies on the limpet *Patella vulgata* as a basis for correlating the scattered raised marine beaches of interglacial age(s) from south-west England and Wales. We show that the epimerisation of D alloleucene: L isoleucene (D allo: L iso) in *P. vulgata*, provides a useful basis for regional correlation. This mollusc lives attached to rocks above the mean tide level and, hence, spends a good portion of its life living at the ambient air temperature. We suggest that the fossil material may have been primarily exposed to regional atmospheric climate since death, because all available evidence points to sea level being lower than present during much of the past 120,000 yr (ref. 6).

Table 1 Amino acid ratios on *Patella vulgata* (*Pv*) and *Macoma balthica* (*Mb*) from sites in south-west UK

Sample no.	Sp.		Location	D allo : L iso (combined)	D allo : L iso (free)
AAL-767	*Pv*	Modern	Saunton	$\bar{x} = 0.014 \pm 0.007(3)$* [min. 0.007]	—
AAL-786	*Pv*	Holocene	Portland	$\bar{x} = 0.04 \pm 0.006(2)$ [min. 0.028]	$\bar{x} = 0.439 \pm 0.06(3)$ [min. 0.379]
AAL-766	*Pv*	Holocene	Skarra Brae	$\bar{x} = 0.04 \pm 0.0015(4)$ [min. 0.038]	$\bar{x} = 0.35 \pm 0.014(2)$ [min. 0.34]
AAL-768	*Pv*		Burtle Beds	$\bar{x} = 0.119 \pm 0.009$ [min. 0.110]	$\bar{x} = 0.705 \pm 0.066(5)$ [min. 0.608]
AAL-769	*Pv*		Minchin Hole Outer Beach	$\bar{x} = 0.099 \pm 0.009(4)$ [min. 0.095]	$\bar{x} = 0.663 \pm 0.068$ [min. 0.59]
AAL-771	*Pv*		Middle Hope	$\bar{x} = 0.101 \pm 0.005(4)$ [min. 0.098]	$\bar{x} = 0.476 \pm 0.088$ [min. 0.38]
AAL-772	*Pv*		Portland	$\bar{x} = 0.125 \pm 0.01(4)$ [min. 0.123]	$\bar{x} = 0.461 \pm 0.039$ [min. 0.41]
AAL-770	*Pv*		Saunton	$\bar{x} = 0.26 \pm 0.066(4)$ [min. 0.2] (0.081; 0.086; 0.09)†	$\bar{x} = 0.629 \pm 0.215(7)$ [min. 0.48]
AAL-839	*Pv*				
AAL-829	*Mb*		Burtle Beds	$\bar{x} = 0.14 \pm 0.025(4)$ [min. 0.12]	$\bar{x} = 0.499 \pm 0.1(5)$ [min. 0.44]

Sites are shown in Fig. 1 and the data are plotted in Fig. 2.

* No. in sample, when >3 this figure includes reruns.

† Five valves including one radically different ratio, for which three reruns are given in parentheses.

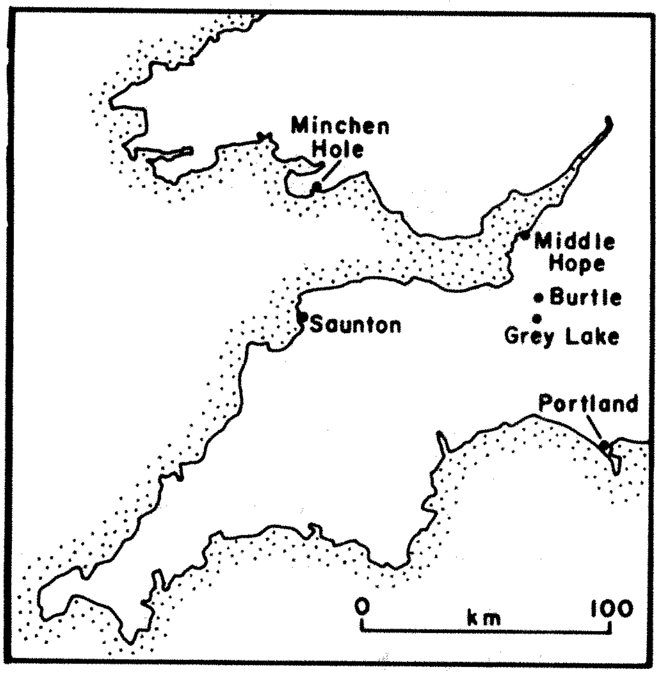

Fig. 1 Sites of interglacial raised marine beaches.

Three shells were prepared from each site (Fig. 1; Table 1). The shells were first cleaned in 2M HCl, and the cleaned fragments dissolved in 8M HCl. Half the aliquot was then dried under vacuum and rehydrated with a solution of *p*H 2 containing a 'spike' of the inorganic amino acid, norleucene. This spike provided an internal calibration. The other half was heated in a controlled oven at a temperature of 110 °C for 22 h. It is then dried and rehydrated. These two preparations provided a measure of the free amino acids (those released by a natural diagenesis) and combined amino acids (free plus peptide-bound). The sample was then placed on an ion-exchange amino acid analyser. For most age dating purposes, we relied on the free and combined ratios of D allo: L iso to indicate the relative age of a sample[1,2]. In addition, we performed pyrolyosis experiments on modern shells of *P. vulgata*. Cleaned shell fragments were heated at 144 °C in the presence of 1 ml purified water. At selected times, the samples were removed from the oven, prepared and analysed (Table 2). The results can be compared to similar experiments on valves of *Hiatella arctica*. A feature of the reaction of *P. vulgata* to the heating experiments is the rapid approach of the free fraction to equilibrium (D allo: L iso = 1.35) and the slow epimerisation of the combined D allo: L iso ratio.

A total of eight shell collections have been processed (Table 1; Fig. 2). They consisted of: a modern valve of *P. vulgata* (AA1-767), two samples of Holocene age from Scotland and south-west England, and five collections from some of the classic raised marine beaches of the region (Fig. 1)[7,8]. The modern shells show no free amino acids and the D allo: L iso for the combined amino acids varies between 0.007 and 0.02. This ratio is caused by the actual processing of the samples. The modern ratios are significantly different from those obtained from Holocene shells (Table 1) where the D allo : L iso (free) averaged 0.35 and 0.44 and 0.04 and 0.032 (combined) respectively.

Four of the five samples from the raised beaches of south-west England and Wales (Fig. 1; Table 1) fall into two groups with D allo : L iso (combined) ratios of 0.10 and 0.12 (Fig. 2). Repeat analyses of the same preparation (=shell) gave precisions of

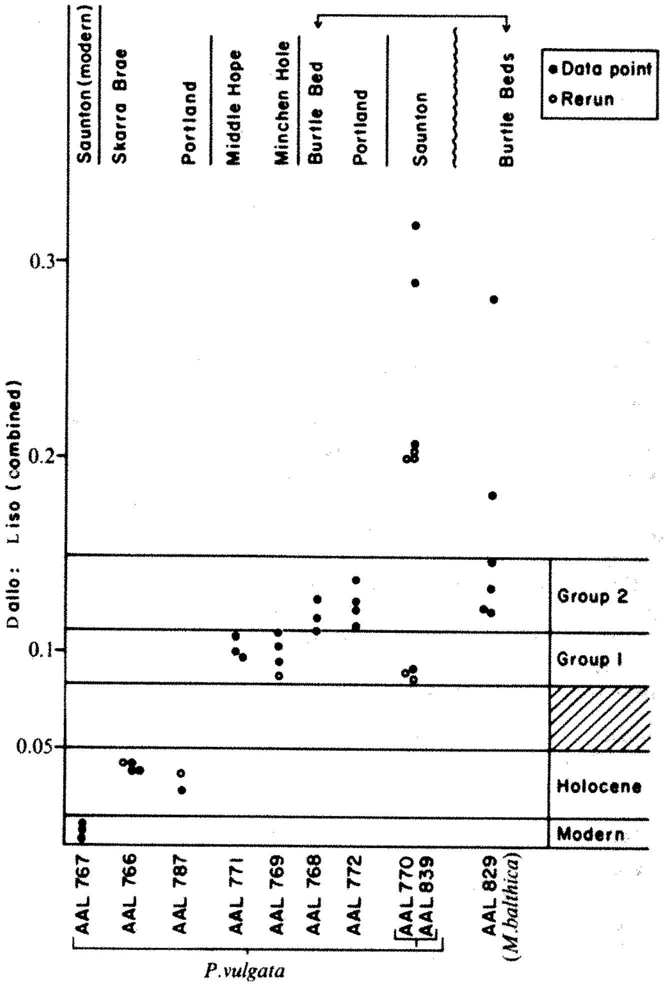

Fig. 2 D allo : L iso ratios for combined amino acids on *P. vulgata* and *M. balthica* from sites in the south-west UK. Data from Table 1.

±5%—smaller than the usual variability between shells from a single stratum (5% to 18%). Analysis of variance (one-way) on the combined D allo : L iso ratios indicates that we can reject the null hypothesis (95% confidence level) that the four samples come from a single population ($F = 8.37$, d.f. 3, 8). The free D allo : L iso ratios (Table 1) do not show an exactly similar pattern and are not considered as reliable an index of relative age as the combined ratio[10,11].

The Saunton raised beach site is not so straightforward in the interpretation of the amino acid ratios. Four out of the five shells gave combined ratios averaging 0.26 ± 0.066 which indicates either (1) that higher environmental temperatures affected these shells since death, or (2) the shells are distinctly older than the same species from the other four sites. The fact that one shell gave a typical value for the other raised marine beaches of 0.1 (combined) may indicate that the beach is the product of two distinct high (interglacial) sea levels, a possibility indicated on stratigraphic sources at Minchin Hole Cove, Gower[7], or that contamination has affected some valves. It is difficult to invoke an explanation for the higher ratios that involves some local temperature perturbation; thus, we favour the second explanation while admitting that more research is required on shell assemblages from the Saunton site. The Portland raised beach and the Burtle Bed samples show somewhat higher combined D allo : L iso ratios than the two other beaches of supposed last interglacial age, taken as equivalent to oxygen isotope stage 5e (ref. 9). It is not yet clear whether the difference reflects a real difference in age or is related to environmental conditions at these sites.

Five valves of *Macoma balthica* were also run from the Burtle Beds (Greylake no. 2). One valve had a cement residue after cleaning and gave a combined D allo : L iso ratio of 0.282. The remaining four valves produced a tight cluster of ratios averaging 0.14 ± 0.025 (Table 1).

Amino acid ratios D allo : L iso for the combined fraction indicate that shells of *P. vulgata* collected from five interglacial sites are either not of the same age, or they have been affected by different temperatures or diagenetic histories since the death of the shells. The Saunton raised beach is clearly the oldest (or most contaminated), although one of the five shells analysed had a ratio similar to those from the Middle Hope and Minchin Hole raised beaches (Fig. 2). These last mentioned sites have ratios somewhat smaller than those determined from the Portland and the Burtle Bed sites. A preliminary conclusion is that the amino acid data suggests the presence in the south-west UK of raised beaches of either two or three different ages. The Portland beach has been regarded as marginally older on altimetric grounds[12]. We cannot yet determine whether these represent sea level highs during whole or part of marine isotope stage 5, or part of that 'interglacial' and an earlier interglacial in the case of the Saunton data (Fig. 2). An alternative explanation of our Saunton data is that the majority of the shells are contaminated and that the age of the beach is given by the ratios of 0.09 (Fig. 2).

Correlation between our sites and those reported by colleagues from south and east England[11] is made difficult because of the absence of *Patella vulgata* in those sites and because our sites generally lack *Corbicula fluminalis*, the species used most extensively in their study. Our *Macoma balthica* ratios, however, provide a start to such a correlation programme. Results from assays of *Macoma* spp. from the crag deposits of East Anglia[11] (Table 3) show significant differences from our findings. The closest site between our data and that of Miller *et al.*[11] is at Selsey, southern England, where a single *M. balthica* gave a combined ratio of 0.33. This ratio is much greater than our results (Table 1), although we note that one sample from our collection which could not be cleaned fully gave a comparable ratio to the *M. balthica* from Selsey. We reject our ratio, however, because we judge it to be contaminated and conclude that the Burtle Beds (Greylake 2) site is characterised by *M. balthica* ratios (combined) of 0.14 ± 0.025.

Table 2 Results of heating *Patella vulgata* at 144 °C

Hours heated	Free (D allo : L iso)	Combined (D allo : L iso)
Control (0 h)	—	0.015
20	0.88 (0.66)*	0.194 (0.292)
45	1.25	0.258 (0.66)
80	1.25	0.427
120	1.26	0.44
232	1.23	0.81
286	1.38	0.83

* Values in parentheses refer to *Hiatella arctica*.

Table 3 Ratios of *Macoma* spp. from southern and eastern UK* compared to Burtle Beds

Site		Combined D allo : L iso
Burtle Beds		0.14 ± 0.025
Selsey		0.33
Clacton		0.58 ± 0.03
Weybourne Crag	East Anglia	0.8 ± 0.02
Scrobicularia Crag	Sites	0.94 ± 0.005 : 0.998 ± 0.18

* Data from ref. 11.

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Abrupt end of the last interglacial s.s. in north-east France

Geneviève Woillard

Chargé de Recherches du Fonds National Belge de la Recherche Scientifique, Institut Botanique, Laboratoire de Palynologie et Phytosociologie, Université Catholique de Louvain, Place Croix-du-Sud 4, 1348 Louvain-la-Neuve, Belgium

Close study of past interglacials might indicate how and when the present interglacial will end and whether we are heading towards a warming or a cooling[1,2]. No certain prediction has been possible because of man's interference with the environment. But, we report here that when exploring the records of past temperate intervals, we observed frequent signs of abrupt changes of local environment. In particular, abrupt shifts in forest composition end each Pleistocene interglacial whose record was studied in detail. In Grande Pile (north-east France), the Eemian (s.s.) (oxygen isotope substage 5e) temperate forest was replaced by a pine–spruce–birch taiga within ~150 ± 75 yr. These results are based on rich pollen content of continuously deposited laminated gyttja and on the assumption of a constant sedimentation rate during the last 11,000-yr long interglacial. If the area today was affected in a similar way, the relatively fast transition of the environment, and by implication of the climate, would place serious constraints on man.

The continuous pollen-rich deposit from the Grande Pile lake gyttja and peat bog, in southern Vosges (north-east France), is very suitable for this kind of study[3–6]. It covers the interval from 140,000 yr BP to the present, and includes the last interglacial,

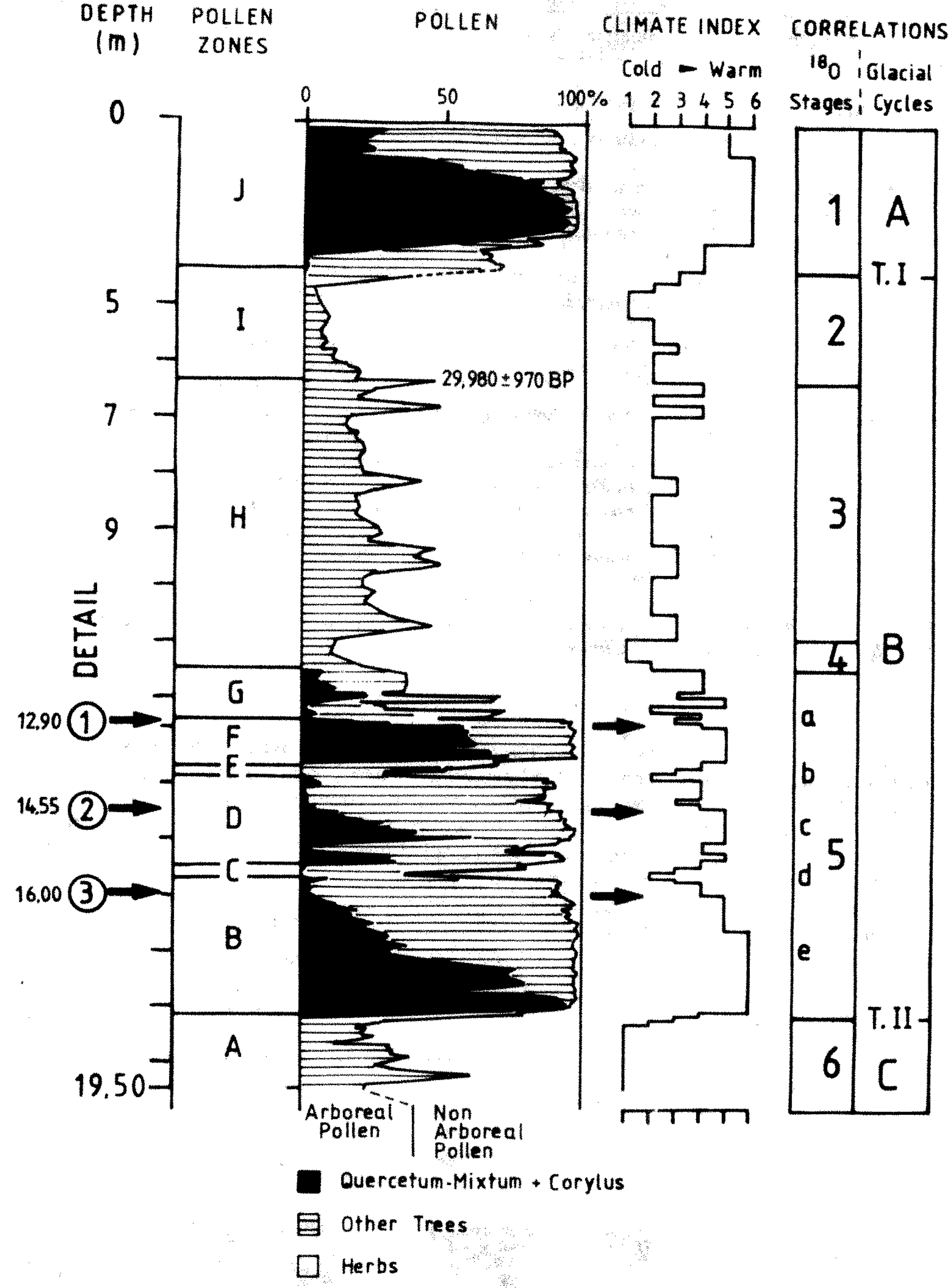

Fig. 1 Grande Pile palynological sequence from the present to 140,000 yr BP. Simplified figure after G.W.[5,6]: general pollen sequence established with the three cores I, XIV and X; zones A to G from the core X. Between 200 to 500 pollen grains counted at 277 levels; percentages based on total sum. Climate index based on variations in pollen assemblage: 1, tundra; 2, shrub-tundra; 3, open taiga; 4, dense taiga; 5, mixed hardwood–coniferous forest; 6, hardwood forest. ^{18}O Stages after Emiliani[27,28] and Shackleton[29,30]; glacial cycles after Kukla[31] and Ruddiman and McIntyre[32]; T. I. (Termination I) and T.II (Termination II) after Broecker and van Donk[33]. 29,980 ± 970 yr BP is the ^{14}C date Lv. 748. Arrows ①, ②, ③ indicate abrupt climatic changes shown in detail in Figs 2 and 3.

the last glacial and the Holocene (Fig. 1). The climatostratigraphic information is directly comparable to that obtained in northern Greece[7,8] and in oceanic records[4,5,9]. Three forest episodes (Fig. 1, zones B, D, F) probably correlate with the oxygen isotope stage 5 (Fig. 1). But, only the lowermost one indicates a climate as warm as the present, and can be correlated with typical pollen profiles of the Eemian, as defined in its type area[10]; it is the equivalent of oxygen isotope substage 5e peaking at 125,000 yr BP.

The deposit is finely laminated, so there is no doubt about the continuity of deposition and about the complete lack of post-depositional disturbance.

An abrupt change of vegetational assemblage is observed to end each of the three forest episodes (Fig. 1, arrows 1, 2, 3): all the temperate species retreat, and are even eliminated by a sudden expansion of boreal trees (Fig. 2). A rapid rise of *Pinus*, followed by *Betula* and *Picea*, ends the *Quercus–Carpinus–Corylus* phase of the upper interval (Fig. 2*a*), the *Quercus–Carpinus* phase of the median one (Fig. 2*b*), and the *Abies–Picea–Carpinus* phase of the lowermost one (Fig. 2*c*). A comparatively abrupt change in vegetation is observed in pollen diagrams from elsewhere, ending past interglacials[7,10–19].

As shown at Grande Pile[5,6], the Eemian is one of the previous interglacials most resembling the Holocene (climate index in Fig. 1, zones B and J; description in Table 1). The transition from the late-temperate zone to the post-temperate zone[20] (Table 1) within the Eemian is rarely demonstrated in terrestrial records because it represents the end of an interglacial. Sediments of this section are often disturbed or removed by glaciation. At Grande Pile, the bog registered the entire Eemian pollen sequence between two glacial episodes (Fig. 1, zones A and C). The abundantly preserved pollen enabled a close study of the critical transition from zone 6a to 6b to be made (Fig. 2*c* and Fig. 3). A 5 cm-long section of organic sediment was taken from a core of Grande Pile, between two levels previously investigated for pollen, and corresponding to zones Eemian 6a and Eemian 6b respectively, (Fig. 2*c*). The selected section was prepared for thin sectioning, following the histological technique developed by Johansen[21]. Sections 250 μm thick, taken at 1-mm intervals along the 5-cm long core, were analysed for

Table 1 Vegetational development and zonation of Eemian and Holocene interglacials at Grande Pile (zones B and J in Fig. 1), and comparisons

Vegetational development	Zonation*	Eemian s.s.†	Holocene†
Tundra		Open *Gramineae–Artemisia* vegetation	
Taiga	IV Post-temperate	7. *Pinus, Picea, Betula* forest (with development of *Ericaceous* heaths)	
		6b. *Abies–Picea* forest (with *Pinus* and *Betula*)	
Hardwood–coniferous forest	III Late-temperate	6a. *Abies–Picea* forest (with *Carpinus, Alnus, Quercus, Buxus*)	Sub-Atlantic: *Fagus* forest (with expansion of *Abies, Carpinus, Ericaceae*, ruderal plants)
		5, *Carpinus* forest (with expansion of *Alnus, Abies, Buxus*)	Sub-boreal: *Fagus, Corylus, Alnus, Quercetum-mixtum* forest (with some *Abies* and *Taxus*)
Hardwood forest	II Early-temperate	4. *Corylus* forest (with *Quercus, Taxus, Alnus*)	Atlantic: *Corylus, Quercetum-mixtum* forest
		3. *Quercetum-mixtum* forest (*Quercus, Ulmus, Fraxinus, Corylus*)	Boreal: *Corylus* forest (with *Quercus* and *Ulmus*)
Taiga	I Pre-temperate	2. *Betula–Pinus* forest (with *Juniperus* and heliophilous herbs)	Pre-boreal: *Betula–Pinus* forest (with *Juniperus* and heliophilous herbs)
Shrub–tundra		1. Shrubs (*Betula, Juniperus, Salix*) and heliophilous herbs	Late glacial

Abies (fir), *Alnus* (alder), *Betula* (birch), *Buxus* (box); *Carpinus* (hornbeam), *Corylus* (hazel), *Fagus* (beech), *Fraxinus* (ash), *Juniperus* (juniper), *Picea* (spruce), *Pinus* (pine), *Quercus* (oak), *Salix* (willow), *Taxus* (yew), *Ulmus* (elm).

* After Turner and West[20].

† After G.W.[3].

pollen, and the results are presented in Fig. 3. One thousand arboreal pollen grains, at least, were counted at each level in three independent counts; percentages are based on the total sum of pollen grains. The time scale used here is based on comparisons with the work of Müller[14] at Bispingen, in northern Germany. Müller counted the annual varves through part of his record and estimated that the entire Eemian interglacial (equivalent of oxygen isotope substage 5e) lasted <11,000 yr. About 9,000 yr elapsed from the start of the *Betula* zone to the start of the *Pinus* zone. The same length of time is assumed to separate the beginning of zone 2 from the beginning of zone 6b in Grande Pile, at a constant sedimentation rate (see the Eemian zones in Table 1). A rate of about 6 yr mm^{-1} thus applies to the sediments of the sampled interval. The maximum error of this estimate may be ±50%.

The pollen data, within the 5-cm section, describe the history of local vegetation as follows (Fig. 3). From bottom to top, four phases can be observed: Zone 6a-top, tree pollen proportion is

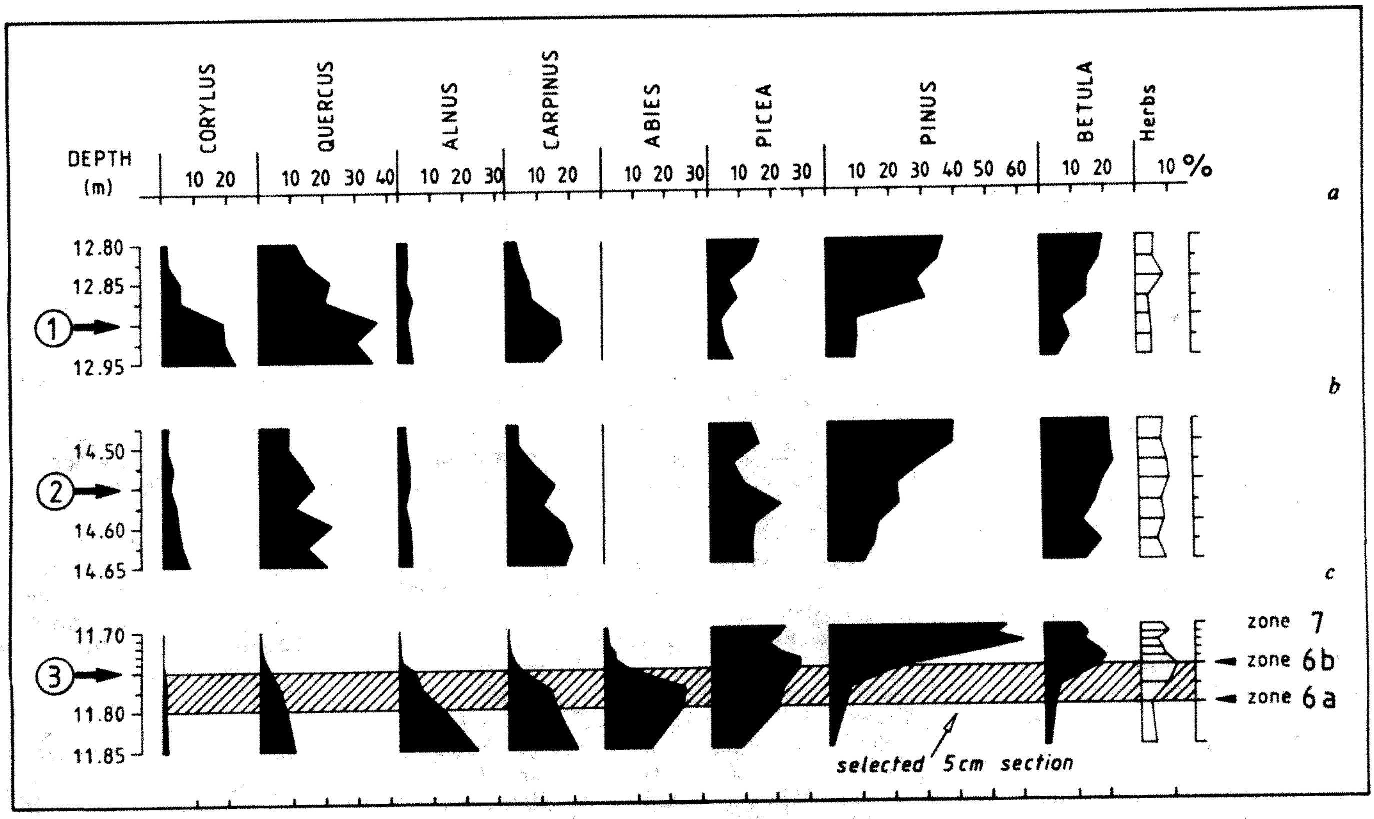

Fig. 2 Pollen counts (% of total pollen sum) across the levels of abrupt environmental change ending the three temperate intervals in Grande Pile shown in Fig. 1. Level 3 was counted in core Grande Pile III instead of Grand Pile X for technical reasons. (Depth level 11.75 in Grande Pile III corresponds to level 16.00 in Grande Pile X, shown in Fig. 1).

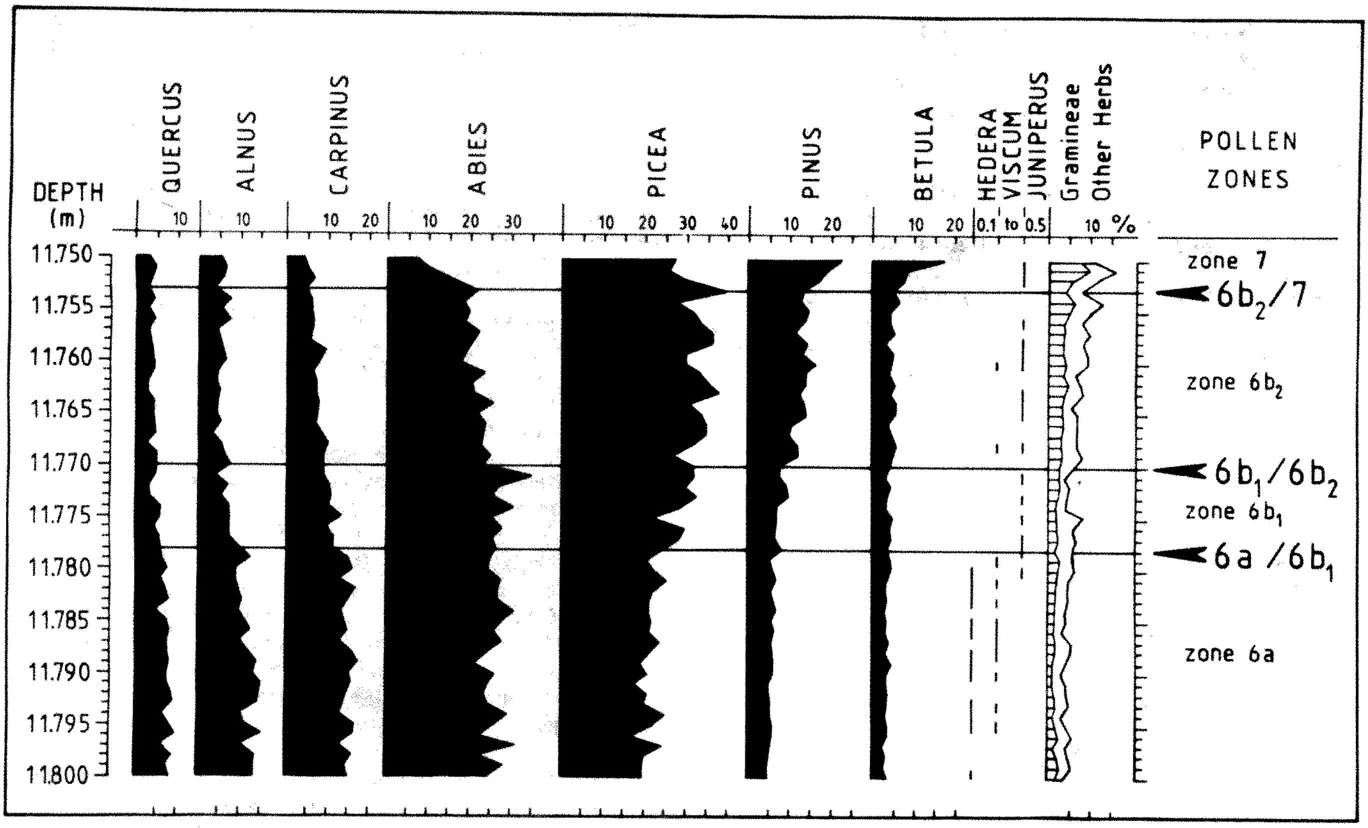

Fig. 3 Selected pollen types in % of total pollen sum. Taken from 250-μm thin sections at 1-mm intervals within the 5-cm segment shown in Fig. 2*c*. Estimated deposition rate is 1 mm per 6±3 yr, so that the segment sampled in detail covers 300 ± 150 yr. At each level 1,000 pollen grains were counted.

regular, with little variation, indicating a stable composition of the forest. Zone 6b$_1$, *Picea* gradually increases, competing with *Abies*: temperate elements decline. Zone 6b$_2$, *Picea* now clearly dominates over *Abies*: temperate elements are almost missing. Zone 7, Sudden expansion of *Pinus* and *Betula* and abrupt decline of *Abies*. (I assume that all the species involved in the competition existed in the pollination area of Grande Pile, within a radius of about 50 km.) Based on the time scale chosen, the upper part of zone 6a (22 mm thick) had a duration of ~130 yr, zone 6b$_1$ (8 mm thick) ~50 yr, zone 6b$_2$ (17 mm thick) ~100 yr, and zone 7 (3 mm thick) about 20 yr. Three relatively rapid changes in vegetation thus appear in the diagram. These are the 6a/6b$_1$ boundary where *Picea* dominates over *Abies* for the first time. Simultaneously, the temperate deciduous trees (*Quercus* and *Alnus*) decline, even the late-migrating *Carpinus*. Then, for ~50 yr, a close competition between *Abies* and *Picea* takes place, the latter gradually expanding. At the 6b$_1$/6b$_2$ boundary, the preceding trends become more pronounced. Now, we can clearly see the dominance of *Picea* and the rise of *Pinus*. The transitional period 6b$_2$ lasted ~100 yr. The main shift in vegetation apparently took place in only a few years, at the level 6b$_2$/7. The 6b$_2$/7 boundary is the best marked in the diagram: in ~20 yr, *Pinus* and *Betula* became with *Picea* the main constituents of the landscape while other trees, even *Abies*, were removed. The landscape is totally modified: the temperate hardwood forest is replaced by a boreal forest, common today in northern Scandinavia between 60 and 65 °N, that is some 15° lat further north than Grande Pile. But, change at the 6a/6b$_1$ boundary also seems to be important: it marks the irreversible decline of temperate elements (Table 1, zones Eemian 6b and 7). What happened at this level? The *Abies* behaviour on the Alsatian slope of the Vosges, during the very dry summer in 1976, might give one possible answer. *Abies* was seriously damaged by the severe summer drought; it was killed at the low altitudes and only survived in cool high areas. Clearly, repetition of such severe droughts would definitely exclude *Abies* from the Alsatian slope of the Vosges. But *Abies* is not the only species affected; all deciduous trees show a decline, even species resistant to drier conditions such as oak. The quasi-continuous presence of *Viscum* (mistletoe) which requires relatively high summer temperatures (+15.5 °C, after Iversen[22]), and that of *Hedera* (ivy) which requires mild winters (temperatures above −1 °C, after Iversen[22]), were ended. At the same time, *Juniperus* became common, a sign of transitions from warm to cold periods. These observations suggest that the change was primarily related to cooling and not to drought.

However, the pollen diagram alone cannot accurately reveal the cause of the change. The pollen curves record local and regional vegetation, and many local or regional factors, unrelated to climate, may modify the landscape[23,24] (soil structure, soil moisture, local or regional soil variations, competition between species, parasitic fungi and bacteria, destructive insects, diseases). Moreover, change of predominant wind direction may influence the pollen rain. Some pollen may be transported over long distances. For example, in the forest–tundra zone of the Nouveau-Québec, Canada, the pine area has its boundary at 55 °N, but the pollen is carried much further north. Heim[25] found 30.8% of *Pinus* in the tundra of Povungnituk, at 60° 34′N. Nevertheless, Heim[26] estimated that 60% of the total pollen represents the local vegetation, only 40% resulted from a regional pollen transport.

In Fig. 3, the change during 6a–6b$_2$ units proceeds gradually, suggesting that the controlling factor was non-episodic. The change at 6b$_2$/7 boundary is more difficult to explain, because it is so sudden. The continuity of sedimentation excludes any hiatus. The major break in vegetation, marked by the sharp rise of *Pinus* and *Betula*, might be due to sudden death of competitive species with higher climatic requirements. A similar replacement of a temperate forest by a boreal forest is observed at the end of all previous interglacials. It is, therefore, reasonable to assume that the sequence, shown in detail in Fig. 3, is typical, and mainly reveals the impact of global cooling on regional vegetation. This means that regional factors may have had an accelerating or delaying effect locally, and that different floral assemblages may have responded differently and at different rates to the climate change.

My pollen work shows that, in Grande Pile, the change from a temperate to a boreal vegetation occurred over 150 yr (±50%). The accompanying gradual decline in temperate elements would probably now be hardly perceptible to man, because of the artificial management of many European forests. We thus cannot exclude the possibility that we already live at the beginning of the present equivalent of the terminal interglacial pollen zone, and that we are heading towards a relatively fast, perhaps dramatic, 'borealisation' of West European forests which, some 115,000 yr ago, took <20 yr.

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Small non-passerine birds of the Lower Tertiary as exploiters of ecological niches now occupied by passerines

C. J. O. Harrison

48 Earl's Crescent, Harrow, Middlesex, UK

In spite of the rather inadequate sample of past avian populations provided by the chance preservation of fossil bird bones, the increasing accumulation of such material makes it possible to speculate on the composition of early faunas and to consider the apparent relative dominance of major groups, such as passerines and non-passerines, in earlier geological periods. An examination of the range of species that we now know occurred in the Lower Tertiary of Britain appears to confirm that passerine birds were very scarce and that their great adaptive radiation had not taken place. Their ecological niches were seemingly occupied by small non-passerine land birds, species of this size appearing proportionally more numerous than at the present day.

Recent studies of British Lower Tertiary birds, involving both new and previously-described (but not always correctly identified) material, have greatly increased the number of species known. The total of British Lower Eocene species from the Woolwich and Blackheath Beds and London Clay of the London Basin, and the London Clay of Bognor Regis, Sussex, has risen from 8 to 31 (ref. 1); six species have been described from the Middle Eocene Bracklesham Beds of the eastern Hampshire Basin[2]; the Upper Eocene avifauna from the Barton Beds of the Hordle area of the Hampshire Basin has increased from 9 to 13 (ref. 3), and five species have been described from the Lower Oligocene Hamstead Beds and Bembridge Marls of the Isle of Wight[4]. Further undescribed species are known from the Lower Eocene London Clay of Essex and the Middle Eocene Barton Beds of the Hampshire Basin. Sixteen orders and twenty-three families are now known to be represented in the Eocene and Lower Oligocene of Britain, and by the standards of modern temperate regions this constitutes a reasonable sample of a local avifauna at this taxonomic level, but lacks the complex adaptive radiation of passerines.

As might be expected from these water-borne deposits, most of the species are waterbirds, and include seabirds. The bone fragments of these species are mostly of moderate to large size and are referable to forms which, for the most part, are within the size range of Recent species in the taxa involved, but tend to be towards the upper end of these ranges. This is not surprising because large bones are more likely to be preserved and be noticed.

Collectors, when working in the field, tend to look for material within a particular size range, and there is apparent evidence of this type of 'search image' bias in the British Upper Eocene material. The earlier collections of Upper Eocene avian specimens[3] consist almost entirely of bones or bone fragments more than 23 mm long and fairly thick; but R. Gardner, while searching for the remains of small mammals in the same Barton Bed deposits that yielded the earlier material, recently collected several fragments mostly less than 10 mm long and 2–3 mm thick. Because these included bones of a new small wader and small passerine-type bird, the remains of small species have clearly been overlooked, and a higher proportion of such species is to be expected from future collecting. In spite of the mainly marine and estuarine origins of these Early Tertiary deposits in Britain, the terrestrial avifauna which has been preserved in them is surprisingly large. The term terrestrial is used here to indicate both cursorial and arboreal land birds, as opposed to species from marine, littoral or lacustrine habitats. The size distribution of these terrestrial non-passerines differs from that of the birds as a whole, as the following list of British forms indicates. In it, except where noted, the birds are from the Lower Eocene. The names of some Recent genera and species are given in parentheses to indicate relative sizes of the fossils concerned.

Falconiformes: one large (buzzard, *Buteo*), one medium-sized from the Middle Eocene (kite, *Milvus*), one very small (pygmy falcon, *Microheirax*); Galliformes: two large from Middle Eocene and Lower Oligocene (junglefowl, *Gallus*), one medium-sized (common partridge, *Perdix perdix*), four small, with one from the Middle Eocene, (from two-thirds partridge size to size of quail, *Coturnix*); Columbiformes: one very small (diamond dove, *Geopelia cuneata*, smallest Recent pigeon); Psittaciformes: one medium-sized (Senegal parrot, *Poicephalus senegalus*); Strigiformes: one a little smaller than the little owl, *Athene noctua*; Cuculiformes: one large musophagid, three tiny cuckoos (bronze cuckoos, *Chrysococcyx*); Coraciiformes: one medium-sized (song thrush, *Turdus philomelos*).

Thus, by comparison with the size ranges of the Recent species in the taxa concerned, and in circumstances where there is a tendency for large rather than small species to be collected, of the eighteen species from these seven orders four were large, four medium-sized, and ten small to very small. In this preponderance of small forms the early avifauna differs markedly from Recent taxa. Although about a third of Recent owl species are small, and about one-sixth of phasianid gamebirds; only about

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14 of 131 cuckoos, 11 of 286 pigeons and nine of 263 diurnal birds of prey come into the latter category. Because most of the small fossils were found at the same sites as medium and large ones there is no suggestion that selective transport of material in a marine environment before final deposition has influenced the size range of specimens; and on present evidence these early terrestrial birds show an exceptionally high proportion of small species.

In Recent terrestrial avifaunas most small birds are passerines, Passeriformes. They form 65% of the terrestrial avifauna of Europe; and in a world study of Recent avifaunas, Slud[5] found an overall ratio of 2:1 between passerines and non-passerines in terrestrial faunas, with a slightly higher ratio in forested than in non-forested regions.

The Passeriformes are very poorly represented in the Lower Tertiary. One species has been described from the Lower Eocene of Britain[1] and another has been identified provisionally from the Upper Eocene. Fisher[6] lists one Lower-to-Middle Eocene species from North America which Feduccia[7] has re-identified as piciform, and four Upper Eocene passerines from France which Brodkorb[8] regards as *incertae sedis*. Brodkorb has also questioned the existence of any passerine birds before the Miocene. At best passerines would form only 10% of the known terrestrial avifauna of the British Lower Tertiary. Because the range of forms includes very large and very small birds, representatives of families in which the present species are almost entirely arboreal, and a number of taxa in which the Recent birds have poor powers of flight, there is no reason to suppose that passerine species, if present at that time, would be any less likely to occur as fossils than these other small species.

Assuming that the known fossils are a reasonable sample of the British Lower Tertiary avifauna, then that avifauna differed from all present ones in having a severe deficiency of passerine species, and in a preponderance of small species among the other orders. Most of these small non-passerines probably fed on seeds or insects and other small organisms, thus occupying the main types of feeding niches of Recent passerines. I suspect that the two differences are linked, and that non-passerines originally occupied feeding niches available for small birds, and were later excluded from these by the adaptive radiation of the passerines. This would in turn have affected the opportunity for survival and adaptive radiation of small non-passerine species.

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Complex systems which evolve towards homeostasis

Ken Tregonning* & Alan Roberts

Department of Physics,
Monash University, Clayton, Victoria 3168, Australia

It is still not well understood how large complex systems (such as ecosystems) come into being or how they persist over long periods of time, although recent studies$^{1-5}$ have clarified certain aspects of the associated stability problems. The occurrence of such systems presents no problem if a teleological assumption is valid—that is, if the variables may be presumed (or compelled) to adjust their mutual interactions in advance, to achieve the desired persistence. But when no such 'foresight' is credible, a real problem emerges; how a given variable can interact will generally be constrained by factors internal to it, independent of the 'needs' of other variables or of system stability. This consideration has prompted studies of 'randomly-interacting' system models, and interesting results have emerged$^{1-4}$ concerning the stability of such models—that is, their capacity to return to equilibrium after a fluctuation. However, the question remains: how did such a collection of randomly-interacting entities arrive at an equilibrium in the first place? We present here results from a simple model using mutually interacting variables. We show how, even on assumptions which make large complex and persistent systems highly improbable initially, they can nevertheless be expected to arise in profusion in the normal course of time development.

To fix ideas, our model is presented as an ecosystem but the field of application is much wider. Such an extension is possible because we have not included specifically biological mechanisms; thus, from an ecological as from any other particular viewpoint, the model is bare and lacking in realistic detail. This is deliberate; we seek the consequences of this very general model itself—the 'mathematical' consequences—rather than those peculiar to the one field of application.

We consider, then, a collection of M species in which the (i)th has an intrinsic increase rate r_i (positive for a producer, negative for a consumer). Its interactions with the other species are fully described by $(M-1)$ 'interaction constants' A_{ij}, and A_{ii} describes its self-interaction. The time-dependent variable $N_i(t)$ is taken to measure its population, population density or biomass.

A simple form for the system's equations of motion was chosen, sometimes called the generalised Lotka–Volterra (GLV) equations, but here referred to as the multispecies quadratic (MSQ) model. (This avoids confusion with other definitions of 'GLV model'.) These equations are;

$$\mathrm{d}N_i/\mathrm{d}t = N_i\Big(r_i + \sum_j A_{ij}N_j\Big), \qquad i = 1 \text{ to } M \qquad (1)$$

To investigate the time behaviour, we proceed as usual (see for example ref. 5). The equilibrium values N_i^* are found as the solution of the matrix equation

$$AN^* = -r \qquad (2)$$

The local stability/instability of the system about these equilibrium values is then determined by the absence/presence of eigenvalues of S with a positive real part, where $(S)_{ij} = \mathrm{N}_i^*\mathrm{A}_{ij}$. However, this stability character has no meaning unless the equilibrium point is 'feasible', that is, unless all the N_i^* are positive[3]. A system which is stable about a feasible equilibrium will be called 'homeostatic'.

In computer studies on this model, we have taken the A_{ij} $(i \neq j)$ as non-zero with probability C (the connectance), and then of constant magnitude g. The self-interaction coefficients A_{ii} and the increase rates r_i were chosen as having magnitude 1. By allowing the non-zero A_{ij} and the r_i to be positive or negative with equal probability, a 'random ensemble' was generated for study.

Initial results for M ranging from 2 to 10 have already been reported[5], and are briefly summarised here. In this model, despite its ecological poverty, the subset of homeostatic systems is reminiscent of real ecosystem behaviour—in particular, in the popularity of predator–prey interactions $(A_{ij} < 0, A_{ji} > 0, r_i > 0, r_j < 0)$, the fraction of consumers tolerated $(r_i < 0)$ and the necessity for nearly all species to be self-regulated $(A_{ii} < 0)$. In all these ecosystem-like properties, the simple requirement of homeostasis picks out a subset very different from the rest of the random ensemble.

But in one important respect, the model fails badly: it gives far too low an estimate of the chance that such a random collection of species will be homeostatic, that is, persistent. This chance lies below 2^{-M}, or, for as few as 20 species, less than one in a million. Thus other mechanisms must be considered, if the model is to yield a plausible likelihood for the occurrence of large systems.

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* Present address: Research Branch, Forests Commission, 1 MacArthur Place, Melbourne, Victoria 3000, Australia.

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Table 1 Results of species elimination

	Elimination method: Random	Elimination method: Natural selective
Maximum no. of eliminations required for homeostasis	50	29
Mean no. of eliminations required for homeostasis	46.7	25
Fraction of sample yielding a non-trivial homeostatic system	24%	100%
Fraction of feasible systems stable	100%	100%
Largest homeostatic system resulting	4	29
Smallest (non-trivial) homeostatic system resulting	2	21
Mean ratio of connectance in homeostatic set to that in random ensemble	0.80	0.99
Fraction of eliminated species which were consumers	0.50	0.826
Maximum fraction of consumers in a homeostatic system	0.33	0.25
Minimum fraction of consumers in a homeostatic system	0.00	0.11
Mean fraction of consumers in a homeostatic system	0.20	0.165
Ratio of mean no. of eliminations to initial no. of unfeasible species	1.82	0.99
Final range of values of g	0.88–1.25	0.33–0.39

Sample of 25 from a random ensemble of self-regulated species collections ($A_{ii} < 0$ for all i), with $M = 50$ (25 consumers, 25 producers), $C = 0.2$, $g = 0.25$ initially. Starting with inhomeostatic systems of 50 species, species are successively eliminated and the interaction strength 'scaled' (see text), until a homeostatic system is achieved. In the random elimination method all species have equal chance of removal. A 'non-trivial' system is one containing 2 or more species.

One might think that specifically biological mechanisms now need to be invoked—for example, the evolutionary processes which lead to what Wilson has called 'adaptative equilibrium'[6]. However, we report here a study which invokes no such 'external' consideration, but derives the higher likelihood desired from the bare model itself. This is achieved as follows: Either of the defects which imply inhomeostasis—unfeasibility, or instability—can be expected to result in the eventual disappearance of one or more species, so that the original collection does not persist in time. Furthermore the elimination of species might proceed until only a handful of species remain, perhaps none at all; but alternatively, the residual system might be comparable in size to the original collection. We have tested for this latter possibility, generating random assemblies as described above, but imposing $A_{ii} = -1$ always to ensure the possibility of homeostasis[5].

Starting with a random (almost always inhomeostatic) collection of 50 species, if it is unfeasible we eliminate the species with the most negative equilibrium value N_i^*. A second criterion was adopted to choose the species most likely to disappear from a feasible but unstable collection; we omit this criterion, for reasons evident below. Both criteria are intuitively appealing, and can be supported. The resulting collection of 49 species is then examined. If it is still inhomeostatic, another species is eliminated using the same criteria; this procedure is repeated until a homeostatic system is obtained, or all species disappear. (On reducing the number of species, say to M', we increased the interaction parameter g to g' so that $(M'-1)^{1/2}g'$ kept its initial value $(M-1)^{1/2}\,g$. For the rationale of this 'scaling' (note that it makes homeostasis harder to achieve in the smaller system) see refs 2 and 5.)

Table 1 gives detailed results for a sample of 25 assemblies treated as described. It will be seen that inhomeostasis, whether initial or final, always stemmed from unfeasibility; feasible systems were always stable. (Other evidence for this finding in the kind of parameter range studied already existed, though for a less general model consisting of producers only[3].)

The major result is that no assembly 'collapsed' to fewer than 21 species, and one retained 29. The mean number of species remaining in the final homeostatic system was 25, or half the original number.

The large size and complexity of the final system depended on the elimination being 'natural-selective', that is, designed to mirror the actual result of development in time. This is readily seen from Table 1, by comparing the results with those of 'random' elimination, where such a rationale was lacking; the largest homeostatic system then achieved held only four species, and most of the species had no interspecific interactions.

In the homeostatic systems arrived at by selective elimination, the distribution of interactions was examined; it was not detectably different from that of homeostatic systems obtained in random choice from the ensemble. Moreover, the connectance C remained essentially unaltered in the passage to homeostasis. Thus, if we want to construct a typical large homeostatic system, of size $\sim M$ and connectance C, it is quicker to generate an assembly (almost certainly inhomeostatic) of $2M$ species with connectance C, and let it achieve homeostasis by natural-selective elimination. The alternative is to continue generating assemblies of M species until a homeostatic one appears; we estimate that, for M as small as 25, the elimination method is quicker by a factor exceeding 500,000.

It is evident that, in the natural evolution of such assemblies if randomly generated in time, almost all large, multispecies systems will be those arrived at through elimination. Moreover, they will appear with far greater speed, and thus with far greater likelihood, than would be suggested by the (tiny) proportion of the random ensemble that they constitute.

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Heart attacks and geomagnetic activity

E. G. Knox, E. Armstrong & R. Lancashire

Department of Social Medicine, University of Birmingham, UK

M. Wall & R. Haynes

West Midlands Regional Health Authority, Birmingham, UK

Malin and Srivastava[1] reported a remarkable correlation between daily variations in the geomagnetic field strength and daily admissions to the cardio-thoracic wards of hospitals in Hyderabad and Secunderabad, for cardiac emergencies, during 1967–72. We have now carried out a similar enquiry in the West Midlands region of the UK for the years 1969–70, but were unable to confirm the Indian results.

The medical data consisted of daily admissions for acute myocardial infarction to all hospitals in Warwickshire, including Coventry, Birmingham, Solihull and Sutton Coldfield, during a period of 720 days beginning 1 January 1969. The data were extracted from the Hospital Activity Analysis records of the West Midlands region. The geomagnetic data consisted of the daily sums of the 3-hourly 'Kp-values' obtained from catalogues of recordings taken in the UK[2]. Fluctuations at three geographical sites were reported and agreed closely; the data used in the analysis were those recorded at the station at Hartland,

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UK. There were 6,298 hospital admissions over a 720-day period, a mean of 8.75 per day. The 'Kp-sums', also consisting of integer values, averaged 15.73 per day. Analyses were carried out with both the raw data (n) and logarithmic transformations ($\log_{10}(n+1)$).

Same-day correlation coefficients were calculated between the two values within each of 24 successive 30-day periods. Serial correlations were calculated, separately for geomagnetic activity and for heart attack admissions, between pairs of readings separated by 1, 2, ... 31 days. Finally, over the full 720-day period, correlations were calculated between geomagnetic readings on day 0, and hospital admissions on days −15, −14, ... +14, +15 days.

The results were qualitatively similar for the raw and the log-transformed data. Results reported below refer to the log transforms. The serial correlations of the admission data gave significant values (> 3 s.e. from 0) at 7 days ($r = +0.15$), 14 days ($r = 0.16$) and 21 days ($r = 0.12$). A complex day-of-week variation over the full period ($\chi^2_6 = 67.8$), mainly characterised by a deficiency of admissions on Sundays, confirms the validity and demonstrates the origins of this finding. The serial correlations of the geomagnetic data gave significant positive results (> 3 s.e.) at 1/2 days ($r = 0.50$ at 1 day), 16/17/18/19 days (0.17 at 17 days) and 25/26/27/28 days (0.21 at 27 days). The 27-day cycle represents the period of rotation of the Sun. None of the 720-day-period correlations with intervals of −15, ... 0, ... +15 days showed significant positive or negative correlations; the greatest absolute value was $r = +0.06$ (1.46 s.e.). None of the intra-30-day-period same-day correlations between medical and magnetic data gave a significant value, either positive or negative.

Despite the fact that our methods and materials were sensitive enough to detect serial correlations within each of the individual data sets, our examination failed to confirm the Indian observations. Although there is no clear explanation of the difference between the two investigations, three hypotheses should be considered: (1) heart attacks may have different aetiologies in the two countries, (2) the two sets of electrocardiographic equipment may differ, the Indian equipment being susceptible to external magnetic fields, thus affecting the relative likelihoods of different diagnoses, (3) temporal fluctuations in the Indian medical data, with a period differing from our own 7-day cycle, and due to different social customs, may have interacted coincidentally with one or other of the periodic variations observed in the geomagnetic field.

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Perception of illusory movement

Alex Fraser & Kimerly J. Wilcox

Department of Biological Sciences, University of Cincinnati, Cincinnati, Ohio, 45221

Intensive studies of visual illusion have rarely shown examples of polymorphic responses[1–3]. We show here that, using figures consisting of stripes shaded from dark to light, arranged in repeating sectors, an illusion of movement can be induced in about 75% of observers when viewed peripherally. The responses of the viewers fall into four categories. This polymorphic response suggests a genetic origin.

Figure 1 shows the figures used during the experiments. The four categories of response into which viewers could be divided were: (1) N, seeing no movement, or a slight, inconsistent, jerky motion; (2) DL, seeing smooth continuous movement in the direction from dark to light shading; (3) LD, seeing smooth, continuous movement in the light to dark direction; and (4) V, sometimes seeing smooth, continuous movement in the DL direction, sometimes in the LD direction. The individuals of the N class who see a slight, jerky movement consistently see this in the DL direction. A sample of 678 subjects showed 24.9% N, 59.0% DL, 6.5% LD and 9.6% V. The subjects were tested with two figures in which the shaded sectors were arranged in a spiral and the polarity of the shading was opposite. Sensitive subjects see the illusory movement in opposite directions in the two figures. Care was taken not to bias the responses of the subjects who were simply asked to give their responses to the figures, no mention being made of motion, rotation, and so on. Reaction to the figures in repeated tests separated by intervals ranging up to 2–3 years is remarkably constant, that is, the classification has a high repeatability.

The polymorphism of response suggested a genetic explanation and, therefore, a parent–offspring survey was made of individuals in 83 families. The LD and V classes were grouped to give the data shown in Table 1. There are no differences between sexes nor between reciprocal matings, and the data have been grouped over sexes and reciprocal matings. If we examine the types of matings from N × N to N × DL, to DL × DL, to DL × LD–V, there is a progressive decrease of the frequency of N progeny. There is a clear parent–offspring correlation, indicating that there is either a strong genetic causation or an equally strong familial effect of common environmental factors.

A number of twins were tested to allow separation of genetic and environmental factors. Concordance was computed as the number of twin pairs having the same response divided by the total number of twin pairs. The concordance for MZ twins (29 pairs) was 0.90, for DZ twins (41 pairs) was 0.56, and for non-twin sibling pairs was 0.53 (213 pairs). The concordance for unrelated pairs was 0.40. The differences between MZ and DZ twins are significant, indicating that there is a fairly strong genetic component. Clearly, more data are needed, but the agreement between the parent–offspring and the twin comparisons indicate that the illusory-movement perception (which we are terming the 'escalator' illusion) offers considerable promise for further detailed studies.

If our data are sub-divided by academic disciplines it is apparent that the incidence of the escalator effect differs between academic disciplines. Faculty and graduate students of three departments were tested: Fine Arts, 4.7% N (42 tested), Biological Sciences, 22.5% N (40 tested), Psychology, 56.2% N (42 tested). The Biological Sciences department does not differ from the general expectation of 24.9% N. The other two departments differ significantly from each other, and from the general expectation. Tests of progeny of Fine Arts and Psychology faculty gave the following results: Fine Arts, 5.9% N (17 tested), Psychology, 35.0% N (20 tested). The regression of the progeny towards the population mean of 25% agrees with the genetic determination of the character. These results support the surprising conclusion that genetic differences in

Table 1 Parent–offspring survey of individuals of 83 families

Parental mating type (and no. of matings)	Response type of offspring (%) N	DL	LD
N × N (10)	18 (52.9)	12 (35.3)	4 (11.8)
N × DL (28)	22 (28.6)	43 (55.8)	12 (15.6)
DL × DL (33)	5 (5.1)	77 (77.8)	17 (17.2)
DL × LD–V (9)	1 (3.8)	15 (57.7)	10 (38.5)
N × LD–V (2)	1 (25.0)	0	3 (75.0)
LD–V × LD–V (1)	0	3 (75)	1 (25.0)

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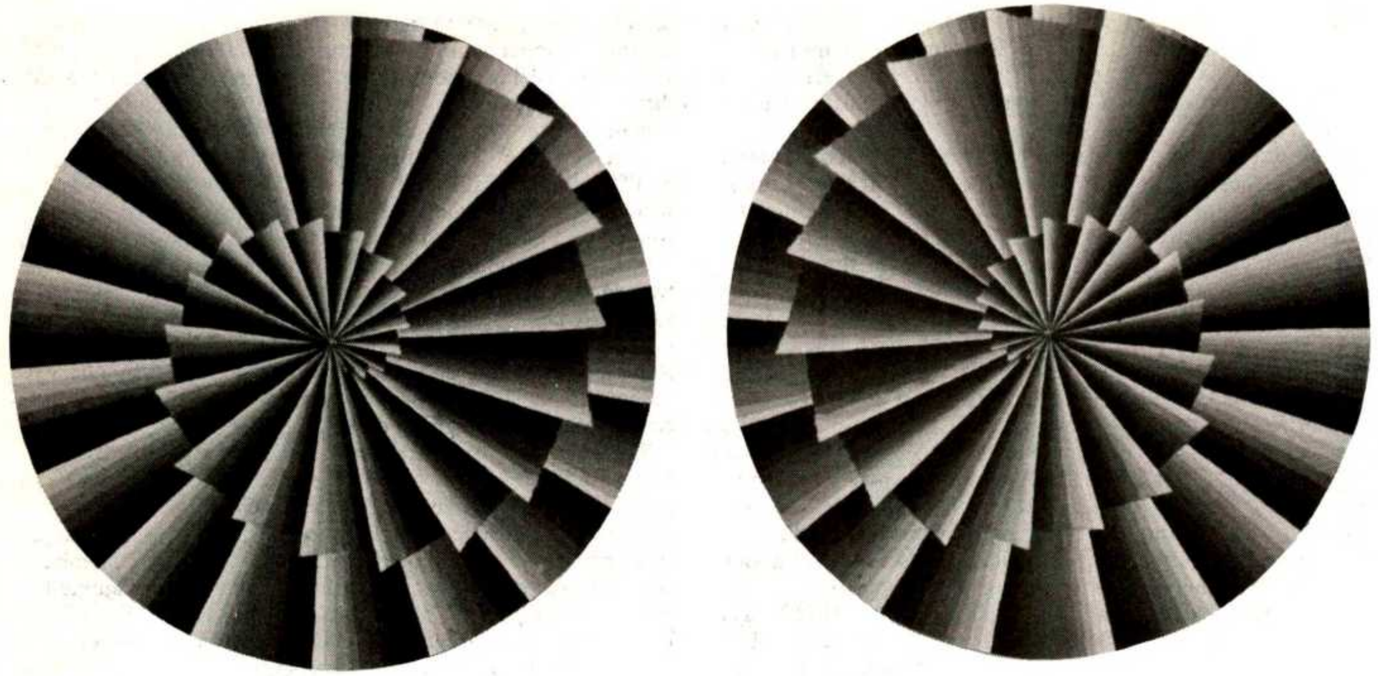

Fig. 1 The two illustrations presented to observers.

sensitivity to the 'escalator' figures are involved in career selection. We have identified in our sample of Fine Arts undergraduate students, five who transferred out of the programme. All were N ($P = 0.001$). Presumably the complementary process occurs in the Psychology programme. Genetic determinism of a sociological polymorphism is known—similar differences between career groups can be found for colour blindness.

'Escalator' illusion is a feature of peripheral vision not occurring within 5° of the point of fixation when seen at distances of several feet. The possibility that this phenomenon is due to small polarised eye movements is discounted, (1) by seeing simultaneous rotation in opposite directions in spiral figures, (2) by seeing simultaneous movement in opposite directions in parallel and rectilinear figures, and (3) by seeing the simultaneous different movements continuously whilst making fairly large eye movements.

Tests were made to determine whether real rotation would suppress or enhance the rotation of the figures induced by the 'escalator' effect. The two figures were mounted side by side and rotated at the same rate. Subjects viewed these figures peripherally centering their vision mid-way between the figures and below them. The two disks were concordant, having their DL shading in the same direction, or discordant, one disk having the DL shading in the clockwise direction whereas its neighbouring disk was shaded DL in the anticlockwise direction. It was found that if the rate of rotation is slow (0.68 r.p.m.) then DL subjects differ in their perception of the rates of rotation of the disks, depending on whether they are concordant or discordant. They see the concordant disks as rotating at the same speed in 91% of trials. Conversely, they see in the discordant disks, one disk as rotating faster than the other. The disk seen as rotating at a faster rate is the one in which the DL shading is in the same direction as the real rotation. This was reported in 68.0% of trials. If the real rotation is reversed, then the identification of the faster disk is also reversed. N subjects gave quite different results in their perception of the discordant pair of disks, reporting the expected difference in apparent rate of rotation in only 18.0% of trials. At slower rates of rotation the agreement of results with expectation increased to 90.0% in DL subjects and to 59.7% in N subjects. At very slow rates of rotation in the range of 0.1–0.4 r.p.m., DL subjects note for the discordant pair of disks that the disk in which the DL shading is in the opposite direction to the real rotation, appeared to be stopped whereas the adjacent disk was still perceived as rotating. Reversing the direction of the real rotation reversed the identification of the disk that was not rotating. N subjects noted the same apparent cessation of rotation only at lower rates of rotation 0.12 r.p.m. and with less consistency. It is apparent that our original test procedure does identify a real difference of visual perception, with the difference being of degree: N subjects are not 'blind' to the 'escalator' effect, but have a much lower sensitivity than DL subjects.

It is rather surprising that the intensive studies of visual illusions[1–3] have rarely identified examples of polymorphisms but a possible explanation of this is that such studies have involved refining figures until they are 'strong'; that is perceived by all subjects in all conditions. If figures are, instead, refined to maximise discrimination, then it is possible that polymorphisms of visual perception will be found to be as general as polymorphisms of other characters.

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Underwater behavioural thermoregulation in the adult stonefly, *Zapada cinctipes*

William Tozer

Division of Entomology and Parasitology, 201 Wellman Hall, University of California, Berkeley, California 94720

Some adult insects survive extremely low temperatures using various physiological mechanisms[1,2]. Here I report that the adult stonefly, *Zapada cinctipes* (Banks) (Plecoptera: Nemouridae), behaviourally thermoregulates at subzero night temperatures by entering the water.

The study site, a small pond formed by an artificial diversion of Sagehen Creek, was located in the Sierra Nevada Mountains 9 km north of Truckee, California (39°25′N, 120°12′W, elevation ~2.0 km).

After typical[3] midday (11.30 a.m.–2.30 p.m.; 13.0–25.0 °C) aerial or surface oviposition (no species of stoneflies in North America have ever been observed to oviposit underwater[4]), a female would generally fly to the centre of the pond and land on the ice. She would then walk to the south-west edge of the ice, crawl into the water and awkwardly swim ~ 0.5 m to within 0.25–0.50 m of the shaded shoreline. Next, she moved beneath the water surface by grasping pine stems and branches protruding above the water. (An adult possesses two pairs of cervical gills and these, in addition to being partially enveloped in a film of air, may enhance underwater oxygen diffusion.) A descending female would wave each antenna in front of her in an apparent searching pattern, although contact was rarely made with the object used for descent. This descent continued to a depth of 15–20 cm, where she remained relatively motionless in the clear water for periods of 20–60 min. (Females placed underwater in wire cages in the same location and raised to a point just below the water surface for inspection at 5-min intervals lived as long as 80 min.) A female would then climb to the surface and float for 1–5 min before submerging again. No females collected before submerging were ever observed to be extruding egg masses. This procedure was repeated continuously up to 5 h after sunset, even after a thin layer of ice had sometimes re-formed on the surface. The outlines of air spaces could be seen immediately under the clear ice cover at irregular intervals along the shoreline. It is within these air pockets that adults are presumed to have obtained surface oxygen, for live adults were subsequently collected on the surface as the ice melted in the early morning. To test for possible oviposition in the night, upper leaf debris and then 2–3 cm of underlying substrate were removed from the pond. Microscopic examination failed to reveal any eggs.

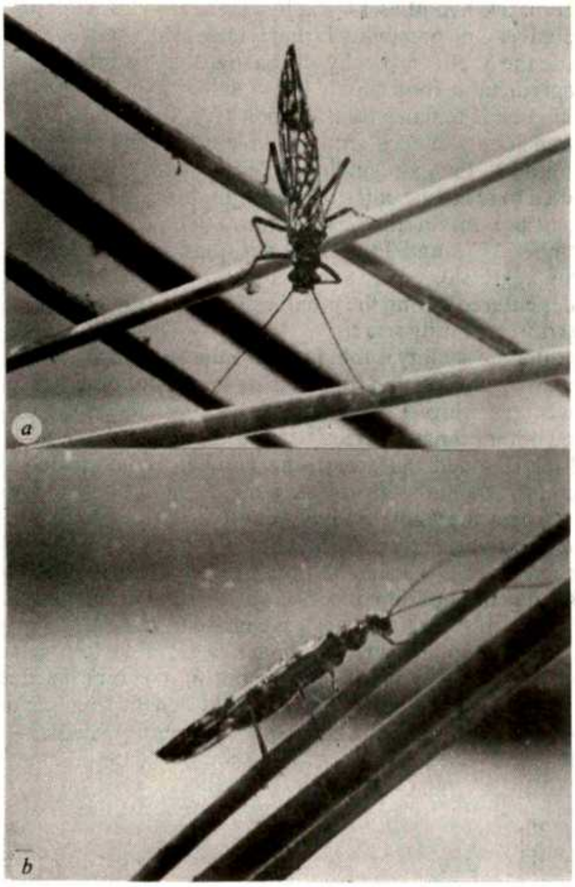

Fig. 1 *a*, *b*, Underwater views of an adult female *Zapada cinctipes* (Banks) after submerging at 5.0 °C air temperature (×2.5).

Such underwater activity was observed for 37 females during 4–18 April 1977. From 19 to 24 April, during late afternoon, adults were more commonly observed to land in open areas along the shoreline and seek refuge in rock crevices and beneath leaf debris (previously inaccessible because of snow cover), where they remained until morning. For the period 4–24 April, mean minimum night air temperature was $(\bar{x}\pm\text{s.e.})-6.2\pm0.7$ °C, and the higher mean minimum water temperature 8.6 ± 1.0 °C.

To test the effect of temperature on survival, 12 individuals collected on 8 April were randomly divided into two groups. The first group was placed in a $0.5\times0.2\times0.1$-m wire cage and half submerged in the pond. The other six were placed in a similar cage, but kept along the shoreline and partly covered against solar overheating. Pond drinking water was renewed daily for the latter group to control for differential mortality from desiccation; gut analyses of both groups (all guts were empty) confirmed an initial assumption that food was made unavailable by caging. Consequently, mean longevity of the first group ($\bar{x}=8.5$ d), with access to the pond, was significantly greater than the second group ($\bar{x}=1.7$ d), which was exposed to subzero night air temperatures ($P<0.005$; Mann–Whitney test).

An additional field experiment was carried out on 21 April 1979 to measure thermal avoidance more directly. To preclude possible light entrainment, this experiment was carried out at times when underwater behaviour has never been observed. (Testing times, air temperatures and water temperatures for both trials were: 10.20–11.05 a.m., 15.0–16.1 °C and 10.0 °C; 1.05–1.52 p.m., 18.0–19.5 °C and 10.4 °C.) The apparatus consisted of a $14.0\times5.4\times14.5$-cm Plexiglass chamber half filled with pond water and submerged in the pond to this same level. Two newly collected females per trial were initially positioned 3.5 cm above the water on pine stems extending the height of the chamber. The top, made of paired sheet aluminium baskets filled with dry ice and separated by an insulated sliding thermistor, was then sealed against CO_2 leakage. At $\sim$6–12 °C, responses consisted of localised repositioning and antennal tip bending and shaking alternately towards the top of the chamber and the water surface. At 4.8–6.1 °C, all stonefly adults moved completely underwater (Fig. 1) for 15–42-min intervals. Adults left the water when air chamber temperatures approached ambient.

A single female submitted to the same experiment at 3.00 p.m., but without access to water, went into a torpor at 3.5 °C. If immediately removed from the chamber and warmed, she recovered and seemed to exhibit normal locomotory behaviour. But, when left at the same temperature for 2–3 min she did not recover.

It seems important that at high altitudes where daily air temperatures fluctuate greatly[5] and winter conditions are severe, endothermic or ectothermic regulation is used by adults as an adaptive survival strategy. Because endothermy for small insects is too energetically costly[6], regulation of body temperature by stonefly adults moving to a relatively constant thermal environment (a pond or lake) may be of considerable evolutionary significance. Such a hypothesis is not without support, for in nearby Lake Tahoe both nymphs and adults of the stonefly, *Capnia lacustra*, have been found to live at depths of 60–80 m (refs 3, 7). Although the behavioural flexibility exhibited by such species seems rather remarkable, limits imposed by poor dispersal abilities[3,8] must be seriously considered in the light of man's increased impact on these faunal refuges.

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Female transference and mate choice among Tana River red colobus

C. W. Marsh

Department of Biology, Universiti Kebangsaan Malaysia, Jalan Pantai Baru, Kuala Lumpur, Malaysia

Red colobus are one of a small number of primate species in which females have been reported to transfer between breeding groups more commonly than males[1-3]. Several authors have hypothesised that in such species transference may serve to reduce the risk to females of producing offspring of lower fitness through inbreeding[1-6]. The hypothesis offers no explanation of why females rather than males are responsible for outbreeding in these species, but remains plausible so long as male membership of breeding groups is relatively stable; for once members of one sex have evolved dispersal mechanisms reducing the risk of inbreeding, pressures on the other sex to do likewise will be lowered[5]. Hence, if both sexes commonly migrate, the hypothesis is weakened. I describe here the membership dynamics of a group of Tana River red colobus, *Colobus badius rufomitratus*, which provide the first evidence of high rates of membership turnover by both sexes in primates, and speculate that the function of female transference in this case may be related to mate choice and the avoidance of infanticide.

The data come from a 3-yr study carried out between 1973 and 1975 near Wenje, in Eastern Kenya. The habitat consists of small patches of riverine forest, differing markedly in size, diversity and seasonality from the rain-forest habitats where red colobus are more widely distributed[7]. The social structure of the Tana population also differs from that reported from rain-forest sites in Tanzania and Uganda[8,9]: instead of multimale groups of about 50 animals, the population is dispersed in groups of 12–30 animals, often containing only a single adult male and never more than two.

Reliable censuses of the main study group were obtained in 24 months between June 1973 and October 1975. During this period the size of the group varied from 16 to 33 animals, with a mean of 21.5. Only a single adult male was resident in the group at one time but there were two replacements during the study. The first occurred in July 1973 and was not observed, and the second took place 5 months later over a 3-day period in January 1974, after which the new male remained for at least another 21 months until the end of the study.

Changes in the number of adult females in the group are shown in Fig. 1. Net gains and losses seem not to have occurred in random sequence (runs test, $P < 0.05$ (ref. 10)), but rather to have involved a rapid decline, followed by a slow increase. The point of reversal in the trend coincides closely with the replacement of the second male in the group. When he joined in July 1973, the group contained 14 adult females and 4 clinging infants. By December, there remained only 8 females, 7 of which carried infants: at least 4 infants had been born in the interim, and one had died. Evidently, in this period pregnant females or those carrying infants tended to remain in the group, while those without young emigrated. On the other hand, after the second replacement in January, female numbers slowly increased to reach a maximum of 19 adults and 3 subadults by the end of the study. With only one exception the increase in adults was due to immigration rather than to the maturation of subadults within the group.

Evidence from known individuals on the origin of immigrants or the fate of emigrants is scarce. Of 11 individually recognisable females, 3 disappeared from the group and 2 were sighted elsewhere. One successfully transferred to a neighbouring group and remained there for at least 16 months, while the other was seen only once in a group some 2 km from the edge of the study group's range. As solitary females were sighted only three times during the study, while solitary males were seen quite commonly, females probably transferred rapidly between groups.

These findings are of interest for three reasons. First, the rate of female movements was extremely high: a total of 34 were inferred over 29 months. Second, the migrations were made by adults 27 times and subadults 7 times, but never by independently-moving juveniles (although two moves were made by adult females carrying small juveniles). Among red colobus in Uganda, females also transfer between groups, but in this case juveniles are the mobile element and the migration of adults has not been reported[3]. If migration served simply to avoid inbreeding, transference at juvenile or subadult age would suffice, and the frequent migration of adults would not be expected. Third, the movements occurred despite two male replacements in the group. Again, if their function was to reduce the risk of inbreeding, they would seem to have been superfluous (unless, conceivably, successive males in the group were closely related individuals).

Alternative hypotheses to explain these findings are at present speculative. One possibility is that females shift between groups because the habitat is patchy and changeable, and thus provides an unpredictable food supply (for a similar hypothesis in birds, see ref. 11). If females transfer, however, because variation in the food supply in each group's range has a critical bearing on their fitness, then on finding a 'good' site, they should be expected to resist the entry of others. In this case the number of females present in the group more than doubled between December 1973 and July 1975, yet there was no evidence of serious aggression against newcomers. Moreover, at least 3 females entered during the months of June and July 1974, when food was apparently scarce[7].

Another possibility is that females migrate because the males in harem groups remain for differing lengths of time, and their replacement brings a risk of infanticide, as may be the case in several other primate species[12-15]. During the 5 months after the third male joined the group, the number of infant deaths in relation to the numbers at risk was 3.1 times higher than the average over the whole study. Four out of seven infants present at the time of his entry died, and one emigrated with its mother. Another, born 2 weeks after the replacement, died within a week, after falling from a tree during or just before an unusual fight in which several females gave chase to the male.

In these circumstances, if females were able to assess the ability of a male to hold a harem during the time needed to conceive and rear an infant, they would benefit from choosing among neighbouring males before becoming pregnant and resident with one. The trait would then be expected to spread

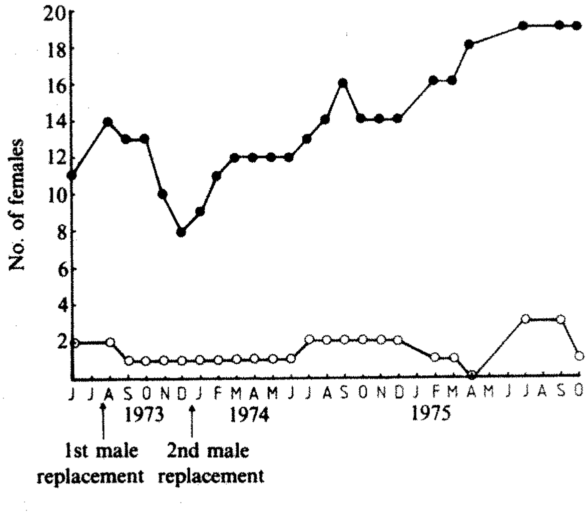

Fig. 1 Variation in the number of females in the Tana study group over 29 months. Also shown is the timing of two male replacements in the group. ●, Adult; ○, subadult.

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rapidly in the population[16]. Such factors could account for the apparently greater preference by females for the third male in the study group, who was resident for at least 21 months, than for the second who remained for only 5 months. Further study of the problem would include a careful comparison of the behaviour of males to detect cues by which females might discriminate between them.

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Captopril (SQ 14225) depresses drinking and aldosterone in rats lacking vasopressin

I. W. Henderson, Annette McKeever & C. J. Kenyon

Department of Zoology, University of Sheffield, Sheffield, UK

Angiotensin II is dipsogenic, and vasopressin (ADH) regulates renal water excretion. Together, these hormones govern overall mammalian water balance[1,2]. The Brattleboro rat with inherited diabetes insipidus (DI) lacks ADH and is therefore a convenient model with which to elucidate mechanisms regulating water metabolism. In the present studies, angiotensin II has also been removed from DI rats by the administration of an inhibitor (captopril, SQ 14225; D-2-methyl-3-mercaptopropanoyl-L-proline) of the enzyme which converts angiotensin I, the relatively inert component of the renin–angiotensin system, to angiotensin II, the biologically active substance[3]. SQ 14225 reduced the drinking rates, and after 6 days lowered peripheral plasma aldosterone concentrations were associated with hyperkalaemia. We conclude that the polydipsia of diabetes insipidus partly results from elevated plasma renin activities and angiotensin II concentrations seen in this syndrome. Further, the apparent hypoaldosteronism of DI Brattleboro rats reflects differences in both tissue usage of the steroid and adrenocortical sensitivities associated with polyuria, hyperosmolarity and possibly potassium wasting[4,5].

DI rats drink approximately eight times more water than normal animals[6,7], and in the present experiments each drank about 80 ml per 100 g body weight per 24 h during the initial 3-day control observation period. When the orally active SQ 14225 was introduced into the water (30 mg l^{-1}), the drinking rates were reduced by about 20% (Fig. 1). Control DI rats with a continuous supply of tap water drank at a consistent daily rate (76.4 ± 1.7 ml per 100 g per 24 h; $n = 5$) over 9 days. The drinking rate was not reduced because rats find SQ 14225 distasteful, as a daily stomach load of water containing the inhibitor lowered drinking rates to a similar extent (unpublished observations).

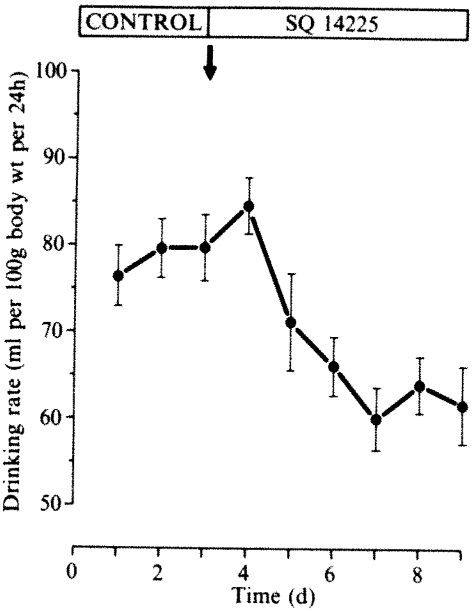

Fig. 1 The effects of SQ 14225 on drinking rate in male DI Brattleboro rats over a 9-day observation period. Rats were isolated for a 3-day adaptation period. Daily water consumption was measured for a further 3-day control period; after this, SQ 14225 was added to the drinking water (30 mg l^{-1}) at (↓), and drinking rates measured for a further 6 days, after which the animals were killed and blood collected. Each point is the mean±s.e. of fluid intake of five rats. Values on days 7, 8 and 9 statistically different at $P < 0.05$. A control group of rats not given the drug at all, gave a drinking rate of 76.4 ± 1.7 ml per 100 g body weight per 24 h. Food intake and growth rates in control and SQ 14225-treated rats were similar.

Peripheral blood was collected from the abdominal aorta under ether anaesthesia on the morning of experimental day 10 (Fig. 1). Plasma osmolarities, sodium and corticosterone concentrations were similar in control and SQ 14225-treated rats (Table 1). Aldosterone concentrations were 66% lower in the SQ 14225-treated rats and plasma potassium concentrations slightly higher than control. Electrolytes were determined by flame photometry, osmolarities by freezing point depression and corticosteroids by radioimmunoassay[8].

Inhibitors of enzymes responsible for the conversion of angiotensin I to angiotensin II have recently been used as potent antihypertensive drugs[9–11]. In particular, those such as SQ 14225 reduced the blood pressure in renal hypertension as well as in hypertension which is free of overt abnormalities in the renin–angiotensin system. Their actions are highly complex, as angiotensin II, in addition to its dipsogenic actions, has feedback relationships with ADH[12], preferentially influences adrenocortical aldosterone biosynthesis, acts on the renal vasculature and has fairly specific roles in regulating blood pressure[2,13].

The severity of the polydipsia and polyuria of diabetes insipidus is attenuated by adrenocortical steroids; water intake and urine production rates fall by 50% after adrenalectomy of DI rats[6]. The present observed reduction in aldosterone concentrations without changes in corticosterone may indicate that the mineralocorticoid itself affects voluntary water intake either by direct actions on dipsogenic centres or indirectly as a result of alterations in sodium appetite and/or potassium balance[14]. The hypoaldosteronism, hypokalaemia and elevated plasma renin activity of DI rats may result from defective renal tubular mechanisms[4], although a possibly increased metabolic clearance rate of aldosterone (R. J. Balment and I.W.H., unpublished observations) could effect similar changes. Indeed, the zona glomerulosa of DI rat adrenals is far more sensitive to angiotensin II than that of non-DI rats[15].

Previous studies indicated that SQ 14225 increased drinking in DI Brattleboro rats[16]; in the same studies angiotensin, rather

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Table 1 Effects of oral SQ 14225 on plasma electrolyte and corticosteroid concentrations in male Brattleboro rats with hypothalamic diabetes insipidus

	Control rats	Rats drinking SQ 14225 for 6 days
Aldosterone	8.4 (5)	2.8† (6)
(ng %)	±2.0	±0.4
Corticosterone	15.9 (5)	12.4 (9)
(μg %)	±2.8	±2.2
Potassium	3.82 (10)	4.24*(9)
(mmol l^{-1})	±0.15	±0.21
Sodium	133.8 (5)	141.6 (9)
(mmol l^{-1})	±3.2	±4.7
Osmolarity	313.8 (5)	307.8 (9)
(mosmol l^{-1})	±6.0	±5.4

Results are means ±s.e. of numbers of rats given in parentheses. * $P<0.1$; † $P<0.02$.

surprisingly, decreased drinking. Direct comparison with the present experiments is difficult, however, as the time course was short (2–6 h), whereas the present data suggest that at least 2 days of treatment are required for the depressed water intakes to become apparent, at least in the doses used. Other investigations have shown that SQ 14225 reduces peripheral plasma aldosterone concentrations in both normal and hypertensive patients[17], whereas in deoxycorticosterone acetate–salt hypertensive rats drinking rates are reduced[18].

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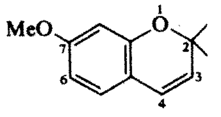
The action of precocenes in milkweed bugs (*Oncopeltus fasciatus*) and locusts (*Locusta migratoria*)

G. T. Brooks, G. E. Pratt & R. C. Jennings

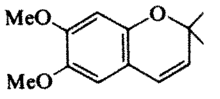
Agricultural Research Council, Unit of Invertebrate Chemistry and Physiology, University of Sussex, Brighton, UK

The active constituents of the plant *Ageratum houstonianum* causing early larval instars of some insects to moult into precocious prothetelic adults are 7-methoxy-2,2-dimethylchromene and 6,7-dimethoxy-2,2-dimethylchromene[1]—precocenes 1 and 2 (P1, P2). P2 causes atrophy of the corpora allata (CA) in at least two species[2–5] and juvenile hormone (JH) production is suppressed, simulating removal of the glands. Active CA are required for the effect of P2 in *Oncopeltus*[5]. The CA are the site of monooxygenase (MO) activity associated with JH biosynthesis[6] and in appropriate conditions, those isolated from certain adult insects can effect the terminal epoxidation of endogenous *E,E*-methylfarnesoate to give C_{16}JH (refs 7, 8). The destructive effect of P2 on the CA, together with the finding[9] that the 3,4-dihydrodiol is a significant metabolite in some insects, suggested that P2 has a specific cytotoxic action within the glands, possibly mediated by local formation of the 3,4-epoxide[10]. Reactive epoxides of polycyclic aromatic hydrocarbons[11], bromobenzene[12], or aflatoxins[13], and oxidative metabolites of other xenobiotics[14] have been implicated in their cytotoxic, mutagenic and/or carcinogenic effects in mammals, in some cases with support from manipulations of the critical balance between activating (oxidising) and deactivating enzymes *in vivo*[12]. We now present evidence that the action of precocenes on immature *Oncopeltus* and *Locusta* requires an oxidative bioactivation within the CA. The generation of reactive metabolites from innocuous precursors in vital tissue, if it can be linked to appropriate species and organ selectivity, offers an attractive approach to insect control.

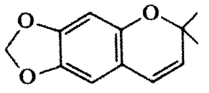
When early second instar *Oncopeltus* were exposed to deposits of various compounds and mixtures in glass jars, P2 at 1 μg cm^{-2} gave total precocious metamorphosis at the 4th instar (Fig. 1), whereas 2H-P2 (compound V, below) had no effect at up to 5 μg cm^{-2}, above which mortality occurred. Thus, the 3,4-double bond is required for biological activity. The methylenedioxyphenyl (1,3-benzodioxole) analogue MDP(III) is also ineffective as an anti-allatin, despite its resemblance to P2 and similar polarity, as attested by an R_F value (TLC on silica gel; solvent, ethyl acetate/xylene, 25:75) lying in the range that includes P1, methylfarnesoate, P2 and C_{16}JH; a relationship which should ensure its reaching the cellular sites, especially those in the CA, that P1 and P2 reach.



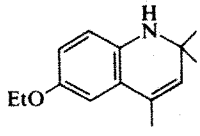
(I) P1*
(IV) 3,4-dihydro(2H-P1)
(VII) 6-methoxy-7H



(II) P2
(V) 3,4-dihydro-(2H-P2)



(III) MDP
(VI) 3,4-dihydro-(2H-MDP)



(VIII) Ethoxyquin

*Compounds except P2 were inactive when tested at 2 μg cm^{-2} as in Fig. 1; P1 is active at 5 μg cm^{-2} upwards.

If an oxidative bioactivation is required for precocenes' action, it is less likely to occur (self-stabilising effect) with MDP, which contains the 1,3-benzodioxole moiety, characteristic[15] of certain insecticide synergists that act by inhibiting MO. Apart from any self-stabilising effect (believed to result in self-antagonism in this case) MDP should also inhibit MO that attack other molecules. Thus, in the cockroach CA bioassay of Pratt and Finney[16], MDP inhibits ($IC_{50} \sim 3 \times 10^{-6}$ M), more effectively than P2 (ref. 17), the epoxidation step in the conversion of exogenous farnesenic acid into C_{16}JH. These considerations make MDP an ideal candidate inhibitor of P2 oxidation in the CA *in vivo* and according to our hypothesis, a likely antagonist of its action. In fact, we find a clear-cut antagonism of P2 action by both MDP (Fig. 1) and its 3,4-dihydro-derivative (2H-MDP; VI) in *Oncopeltus*; normal moulting is restored at

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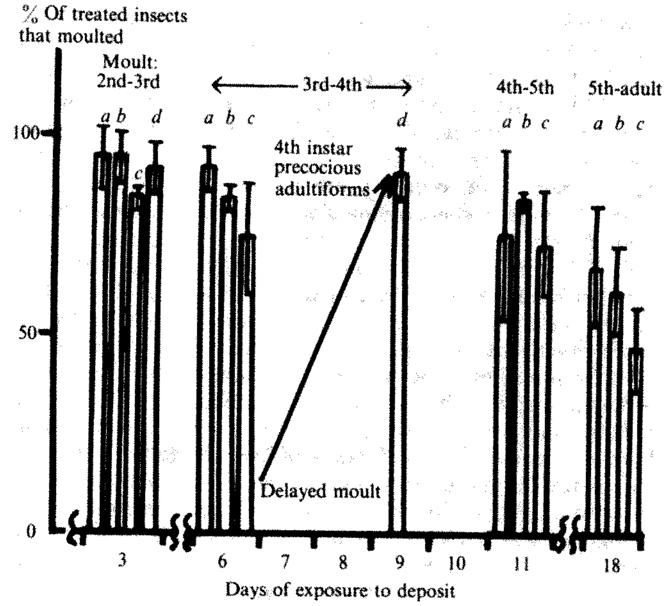

Fig. 1 Groups of 20 early 2nd instar *Oncopeltus* were confined (27°C; 2:1 light/dark regime) in 100 cm^2 glass jars (two groups per treatment) containing milkweed seed and water and surface treated with *a*, impregnating solvent (ether/acetone) only; *b*, MDP only (2 $\mu g\ cm^{-2}$); *c*, MDP (2 $\mu g\ cm^{-2}$) plus P2 (1 $\mu g\ cm^{-2}$); or *d*, P2 only (1 $\mu g\ cm^{-2}$). Except for P2 at the moult 3–4, values (mean of two experiments with range) represent insects in each exposure that moulted normally; others died. With P2 alone (*d*), the normal moult 2–3 is followed by delayed moulting (arrow) to either permanent or short-lived precocious 4th instar adultiforms between days 6–9. When MDP (2 $\mu g\ cm^{-2}$) is co-formulated with P2 (*c*), the normal 3–4 (day 6), 4–5 (day 11) and 5–adult moults are restored.

the 4th and 5th instar with MDP plus P2 (columns *c* in Fig. 1). In some experiments an increased number of deaths (compared with controls) as 5th instar larvae or during the adult moult, indicates a previously noted[18] deleterious effect of P2 at the final moult which is probably unrelated to the anti-allatal effect. However, normal adults arising from antagonism tests with both MDP and 2H-MDP laid eggs that produced viable larvae. MDP showed no JH-activity towards *Oncopeltus* continuously exposed from the late 4th stage to 5 $\mu g\ cm^{-2}$ in the jar deposit test, or when topically applied (400 μg) in acetone to early 5th instar *Locusta*, or (40 μg) to newly moulted pupae of *Tenebrio molitor*.

Compounds such as 2,3-methylenedioxynaphthalene and 2,4,5-trichloropropargyloxybenzene, recognised as 'insecticide synergist' type MO inhibitors[15] gave indications of antagonism with P2 but closer analogues such as P1, 2H–P1 (IV), the P1-isomer(VII), 2H-P2 (V) and the antioxidant ethoxyquin which might inhibit P2 metabolism competitively, had no effect on its action. Thus, efficient antagonists must apparently bear a close structural resemblance to the precocenes and also interact strongly with the oxidase(s) concerned, in the manner of classical insecticide synergists. Sesamex, a JH-mimic for *Oncopeltus*[19], had no apparent effect on development as a 2$\mu g\ cm^{-2}$ deposit but showed partial antagonism in co-formulation with P2; neither peanut oil, liquid paraffin, tocopherol or tocopherol acetate protected against P2 at the 2:1 ratio, thereby eliminating the possibility of a diluent effect in jar deposits. Although sesamex might antagonise P2 action both by inhibiting MO and/or replacing JH, the antagonism is clearly incomplete in these experimental conditions, in contrast to that shown by MDP or 2H-MDP. Neither piperonyl butoxide nor tropital prevented precocious metamorphosis in these experiments.

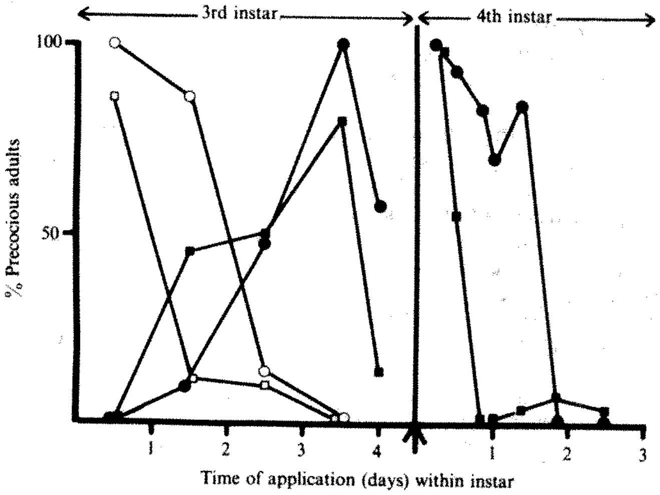

Fig. 2 Periods of sensitivity to the prothetelogenic action of precocene 1, during the 3rd and 4th instars of *Locusta*. We topically applied 50 μg (squares) or 100 μg (circles), in acetone (5 μl), to groups of 10 or 20 larvae at different times during development and recorded the percentage of animals exhibiting precocious metamorphosis at a subsequent ecdysis: hollow symbol, precocious 4th instars; solid symbols, precocious 5th instars. Solvent controls gave only normal animals.

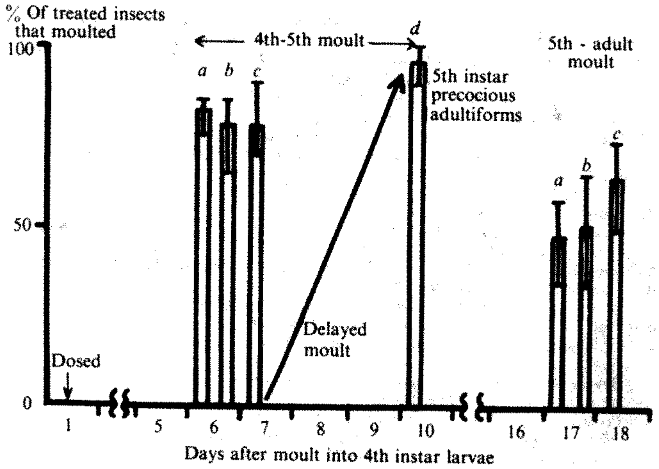

Fig. 3 Groups of 20 recently moulted 4th instar *Locusta* were topically treated with acetone (10 μl) alone, or containing 200 μg of MDP. After 1 h, insects were further treated with acetone (10 μl), alone, or containing P2 (100 μg), to give *a*, solvent control; *b*, MDP only; *c*, MDP/P2 (2:1); and *d*, P2 only test groups which were fed and held in a light/dark (2:1; 40°/25°C) regimen. Values (mean of three experiments with range) represent survival at each moult; precocious 5th instar larvae were not found in *c*, nor normal 5th instars in *d*. The slow moult (arrow) into permanent precocious 5th adultiforms between days 7 and 10 with P2, is prevented by the combined treatment (columns *c*), which gives normal 5th instars and adults.

P1[20] and P2[3,21] cause precocious metamorphosis in *Locusta* and we determined periods of optimal sensitivity to them, as in Fig. 2, which shows that treatment with P1 is effective throughout the 3rd and early in the 4th instar. Whereas P1 and P2 were fully effective at 100 μg when topically applied to early 4th instar larvae, neither 2H-P1 nor 2H-P2 caused precocious metamorphosis at doses up to 400 μg per insect, beyond which toxic effects appeared. As with *Oncopeltus*, the 3,4-double bond seems to be necessary for activity. MDP (200 μg) topically applied 1 h before either compound prevented the effect of 100 μg of P1 or P2. In Fig. 3, P2 applied alone delayed the moult from 4th to 5th instar and produced permanent precocious 5th adultiforms (column *d*), whereas normal 5th instar larvae and adults resulted from the MDP/P2 combination (columns *c*).

Groups of five 4th instar *Locusta*, treated with P2 or MDP/P2 as in Fig. 3, were surface rinsed with ether/acetone (4:1) and the carcasses extracted with this solvent, 3 h after application of P2. Quantitation by GLC showed that up to three times more P2 remained in the insect tissues when MDP was also present, demonstrating that this 1,3-benzodioxole analogue indeed inhibits the oxidative metabolism of P2. MDP can evidently maintain its protective effect within the CA, despite the possibility that more unmetabolised P2 arrives there because of the inhibition of its metabolism in peripheral tissues. Similar effects were observed in studies on the pharmacokinetics of 4-ipomeanol, a toxin that acts on mammalian lung by oxidative metabolism[22].

Earlier work[17] showed that P2 progressively inhibited the final steps, including epoxidation, of C_{16}JH formation from farnesenic acid in the CA of the cockroach, *Periplaneta americana, in vitro*. Further experiments are in progress to test our hypothesis that labile precocene 3,4-epoxides, or derivatives thereof, are proximate insect- and cell-specific cytotoxins (alkylating agents) whose formation in competition with the epoxidation step of JH biosynthesis in the CA of sensitive insects destroys the glands' ability to synthesise the hormone.

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Cell size changes in undeprived laminae of monkey lateral geniculate nucleus after monocular closure

M. P. Headon, J. J. Sloper, R. W. Hiorns & T. P. S. Powell

Departments of Human Anatomy and Biomathematics, Oxford, UK

Transneuronal degeneration in the lateral geniculate nucleus (LGN) due to removal of an eye or closure of the eyelids has traditionally been measured by comparing the sizes of cells in the deprived laminae with those of the corresponding undeprived laminae in the nucleus of the opposite side of the same brain. However, the undeprived laminae may themselves change; some hypertrophy of their cells has been found in kittens following early monocular closure[1] but such comparisons are difficult in the cat because of the variation between animals[2]. In a recent study of the effect of binocular closure in the monkey[3] it was necessary to measure cell sizes in the LGNs of a series of normal monkeys as controls, because there are no undeprived laminae after binocular closure. From this series of normal animals it was apparent that there is much less variation between individual monkeys than has been found with cats and it was thus possible to make reliable comparison of cell sizes between different animals. In the present study the series of normal monkeys has been extended and the mean cell size and its variability between animals have been established for each geniculate lamina in the normal rhesus monkey. We give here a comparison of the cell sizes of both deprived and undeprived laminae following monocular eyelid closure with normal measurements and report some unexpected findings, particularly in animals where monocular closure has been carried out relatively late.

Eight normal monkeys formed the control group, five which were perfused at 8, 9, 17, 32 and 32 days of age and three young adults. In six more animals the lids over one eye were sutured under open ether anaesthesia at various ages and the young monkeys returned to the mother for various periods (Fig. 1). As both these variables have been found to be important the results have been analysed in two groups: early closure, long survival (2) and late closure (4). Details of the methods of fixation, histological preparation and measurement have been described previously[3]. Fifty cells were measured in each lamina of each animal and the sample was taken from the middle of the lateral limb at the junction of the middle and posterior thirds of the nucleus; only cells with distinct nucleoli were drawn. To make comparisons between animals the mean cell area for each lamina in each animal has been treated as a single observation and the averages of all the means and standard deviations of these values have been calculated from these for each group (Tables 1–3).

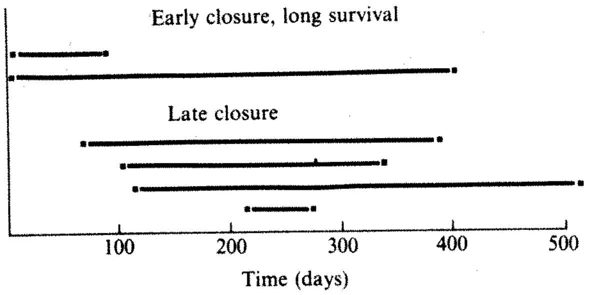

Fig. 1 The age at monocular closure and duration of closure for animals in this study.

Comparisons between groups have then been made for each lamina using Student's *t*-test. Values from the left and right sides of control animals have been treated separately throughout as have the deprived and undeprived laminae of experimental animals. In addition, for each lamina of the normal animals, the range within which 95% of the mean cell areas for a single normal lamina would be expected to fall has been calculated so that individual experimental animals may also be compared with the normal group.

Analysis of the cell area measurements for each lamina in the eight normal animals shows the degree of variability between animals; individual details of five have been published[3] and the small variability of the means from each animal about the group means can be seen from the small standard deviations of the normal measurements (Table 1). The variability in the parvocellular laminae (III to VI) is considerably less than in the magnocellular laminae (I and II). Comparison of the five infants with the three adults shows little or no growth occurring in the

Table 1 Mean LGN cell areas of normal animals (μm^2)

Lamina	Overall	Infant	Adult
	n = 16† s.d.	*n* = 10† s.d.	*n* = 6† s.d.
I	298 ± 26	291 ± 28	309 ± 21 NS
II	294 ± 35	277 ± 30	322 ± 21*
III	196 ± 14	200 ± 12	189 ± 17 NS
IV	195 ± 9	198 ± 10	191 ± 8 NS
V	191 ± 13	191 ± 15	192 ± 8 NS
VI	183 ± 11	180 ± 12	190 ± 7 NS

Significances shown against the adult measurements are for differences from the infant group.

NS, not significant. *, $P > 0.05$, †, Two sides from each animal.

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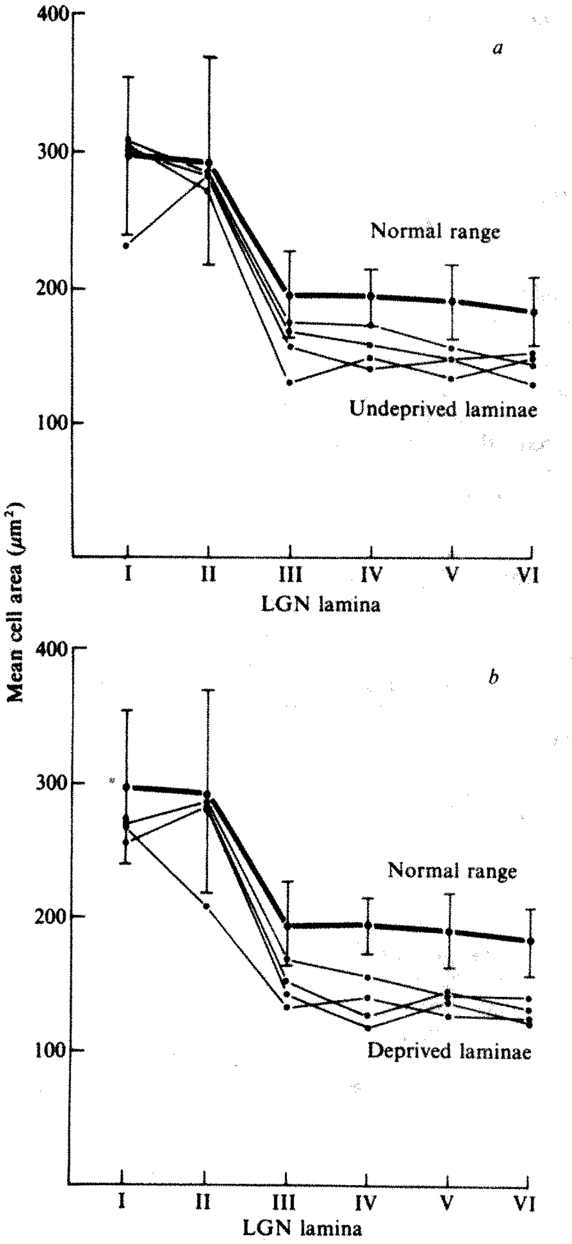

Fig. 2 Mean areas of neuronal somata in the undeprived LGN laminae (*a*) and deprived laminae (*b*) of four monkeys following late monocular closure. Lines join the measurements for an individual animal. Each bar for the normal range indicates an interval within which the mean cell area in a single lamina from one animal may be expected to lie with a probability of 0.95. Most of the values for both the deprived and undeprived parvocellular laminae fall below the normal range indicating shrinkage of cells in these laminae.

parvocellular laminae within the ages studied but there is a little growth in the magnocellular laminae. Measurements of all eight normal animals have therefore been pooled to determine the mean and variability of each lamina in the normal monkey. This variability is much less than that of 'normal' laminae in monocularly deprived animals.

The measurements in the four monkeys with late monocular closures show little shrinkage when the deprived laminae are compared with the undeprived laminae (Table 2). This would be interpreted as showing the decline in sensitivity towards the end of the critical period. However, if these measurements are compared lamina by lamina with those of the normal animals, there is a 20–25% shrinkage of the *undeprived* parvocellular laminae and this is masking a shrinkage of ~30% in the deprived parvocellular laminae (Table 2, Fig. 2). The undeprived magnocellular laminae do not show a comparable effect and the shrinkage of the deprived magnocellular laminae is much less than that of the deprived parvocellular laminae. The differences between deprived and undeprived cell sizes after monocular eyelid closure are thus very similar for the magnocellular and parvocellular laminae. Late monocular closure thus causes a marked shrinkage of the parvocellular laminae but it affects both deprived and undeprived laminae to almost the same extent.

Table 2 Mean LGN cell areas from late closures (μm²)

Lamina	Undeprived	% Change from normal	Deprived	% Change from normal	% Difference between undeprived and deprived
	s.d.		s.d.		
I	286 ± 36	↓4 NS	268 ± 9	↓10*	↓6 NS
II	281 ± 7	↓4 NS	265 ± 38	↓10 NS	↓6 NS
III	157 ± 19	↓20*	150 ± 15	↓23†	↓4 NS
IV	156 ± 14	↓20†	135 ± 17	↓31†	↓13 NS
V	146 ± 9	↓24†	138 ± 8	↓28†	↓5 NS
VI	143 ± 10	↓22†	131 ± 8	↓28†	↓8 NS

NS, not significant.
* $P < 0.01$; † = $P < 0.001$; $n = 4$.

Table 3 shows the mean measurements for the two monkeys with early closure and long survival. Comparison of deprived with undeprived laminae shows shrinkages of 20–30%, in agreement with previous reports. However, if the measurements are compared with those of the normal animals it is apparent that there is a consistent shrinkage of ~10% in the undeprived parvocellular laminae. This means that the shrinkage in the deprived laminae is underestimated by this amount in the traditional method.

As the undeprived magnocellular laminae show little change they form an internal control for the changes in the parvocellular laminae. This may be measured by calculating the ratio of the mean cell area of the magnocellular laminae to that of the parvocellular laminae, taking deprived and undeprived laminae separately. Table 4 shows these ratios for each animal and that the ratio is consistently higher following monocular closure for both deprived and undeprived laminae. In any one animal the ratio changes for deprived and undeprived laminae are remarkably similar, although the mean cell areas from which they are calculated may differ markedly; this indicates that the excess shrinkage of the deprived parvocellular laminae compared with the deprived magnocellular laminae is closely similar to the shrinkage of the undeprived parvocellular laminae compared with the undeprived magnocellular laminae. As well as the generally accepted shrinkage of the deprived laminae in relation to the undeprived laminae following late monocular closure there is thus an additional and larger shrinkage affecting both deprived and undeprived parvocellular laminae equally. The early closure, long survival group shows a similar but smaller shrinkage of the undeprived parvocellular laminae (although the survival periods were comparable in the two groups), indicating

Table 3 Mean LGN cell area of early closure—long survival (μm²)

Lamina	Undeprived	% Change from normal	Deprived	% Change from normal	% Difference between undeprived and deprived§
	s.d.		s.d.		
I	328 ± 21	↑10 NS	243 ± 24	↓18†	↓26‡
II	313 ± 10	↑6 NS	242 ± 20	↓18†	↓23‡
III	179 ± 2	↓9†	128 ± 12	↓35‡	↓28‡
IV	176 ± 17	↓10 NS	131 ± 11	↓33‡	↓26‡
V	172 ± 10	↓10*	122 ± 1	↓36‡	↓29‡
VI	162 ± 8	↓11†	111 ± 19	↓39‡	↓31‡

NS, not significant; *, $P < 0.05$; †, $P < 0.01$; ‡, $P < 0.001$. §, Significance levels in this column are based on the conventional analysis using a comparison of sides within each animal and the standard errors of the 50 cells in each sample are used. Both animals separately achieved the significance level shown for each lamina. $n = 2$.

that this effect increases with age at monocular closure within the age limits of the study.

We suggest, therefore, that the development of the primate visual system occurs in two major phases. Initially the anatomical balance between the connections of the two eyes is established, corresponding to the critical period as presently defined and the period of plasticity of the cortical ocular dominance columns[4,5]; it is during this period that monocular closure may produce hypertrophy[1]. As these connections become established we suggest that the presence of synchronous inputs from the two eyes becomes necessary for the establishment of normal binocularity in the cortex. In the absence of corresonding visual inputs it is no longer possible for both eyes to drive the same cell effectively simultaneously and so the connections from the two eyes interfere with each other and the development of both is inhibited. This interference thus differs from the competition described in the cat during the critical period[6].

Table 4 Ratios of magnocellular to parvocellular cell areas

Normals	Age (days)	Ratio
OM 216	8	1.65
OM 222	9	1.45
OM 225	17	1.26
OM 169	32	1.46
OM 221	32	1.61
OM 185	Adult	1.71
OM 187	Adult	1.68
OM 206	Adult	1.60
	Mean	1.55
Early monocular closure, long survival	Ratio	
	Normal	Deprived
OM 166	1.82	1.85
OM 210	1.90	2.12
	Mean 1.86*	1.99*
Late monocular closure		
OM 200	1.79	1.81
OM 193	2.04	2.12
OM 212	1.83	1.83
OM 194	1.87	1.94
	Mean 1.88*	1.93*

Significances shown are for differences from the group of normal animals.
$* = P < 0.001$.

Three major conclusions may be drawn by comparing the results of monocular closure with those of normal monkeys. First, following late monocular closure or early closure and a long survival period there is shrinkage of the undeprived parvocellular laminae; this leads to underestimation of the degree of shrinkage of the deprived parvocellular laminae if the undeprived laminae are used as a control. The primate visual system is thus more sensitive to monocular closure in older animals than had previously been supposed, with increasing effect on the undeprived laminae. Second, as this effect is restricted to the parvocellular laminae which represent the X-cell system while the magnocellular laminae represent the Y-cell system, these two functional pathways react differently to monocular deprivation. Third, as the cell areas measured in the late closure group for all the deprived laminae and the undeprived parvocellular laminae are smaller than those in normal animals at the corresponding ages of closure it can be concluded in this group at least that actual shrinkage of cells has taken place rather than failure of growth.

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Axonal transport of vasoactive intestinal peptide in sciatic nerve

Antonio Giachetti & Sami I. Said

Departments of Pharmacology and Internal Medicine, University of Texas Southwestern Medical School, Dallas and Veterans Administration Medical Center, Dallas, Texas 75235

Dense plexuses of neurones containing immunoreactive vasoactive intestinal peptide (VIP) have been found in discrete areas of the central nervous system and in peripheral organs, including the gastrointestinal tract, pancreas and urogenital system[1–7]. In many of these locations VIP is concentrated in nerve endings, where it can be released by high K^+ concentrations in a Ca^{2+}-dependent manner[8,9]. VIP release may also be provoked by electrical stimulation of nerves, for example the vagus[10]. VIP thus shows some of the features of neurotransmitter or neuromodulator substances. The presence of immunoreactive VIP in the fine terminal varicosities as well as in the cell bodies of neurones[4,11] suggests that it might be transported from the perikaryon, where it is presumably formed, to the nerve endings, through the axonal transport system[12–14]. Such transport would be in keeping with a role for the peptide as a neurohumor or neurohormone[15]. We report here that VIP accumulates in constricted rat sciatic nerves in a manner suggesting fast, anterograde axonal flow.

Under light ether anaesthesia, the left sciatic nerve in male Sprague–Dawley rats was constricted by tying a silk thread around the nerve below the hip. The contralateral nerve was isolated but not ligated. After 4, 12, 24 or 72 h, the rats were killed by decapitation. The dissected sciatic nerves were cut in consecutive segments (0.5 cm each), beginning with the segment immediately proximal to the ligature. Two or three identical segments from different experiments were pooled, homogenised in 1 ml of ice-cold acetic acid (0.2 M); the homogenates were centrifuged at 8,000 *g* for 20 min and the supernatants were analysed for immunoreactive VIP by a sensitive and specific radioimmunoassay[16]. In four further experiments, the left sciatic nerve was ligated at two points, 1 cm apart; the segment between the two ligatures as well as an equal segment distal to

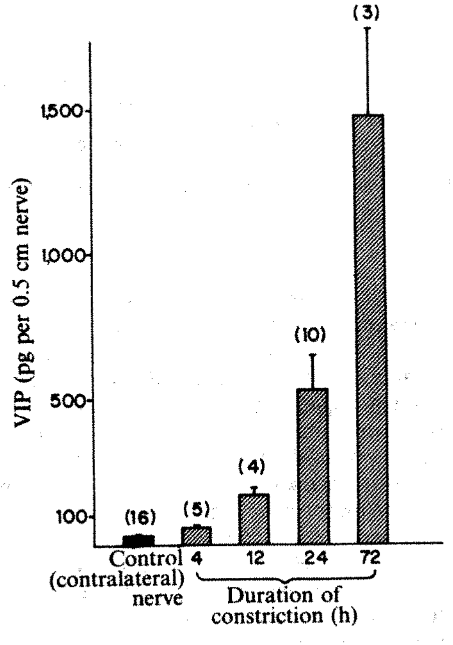

Fig. 1 Accumulation of VIP in proximal segment of sciatic nerve constricted for 4, 12, 24 or 72 h. Values are means ± s.e.m. Number of determinations in parentheses.

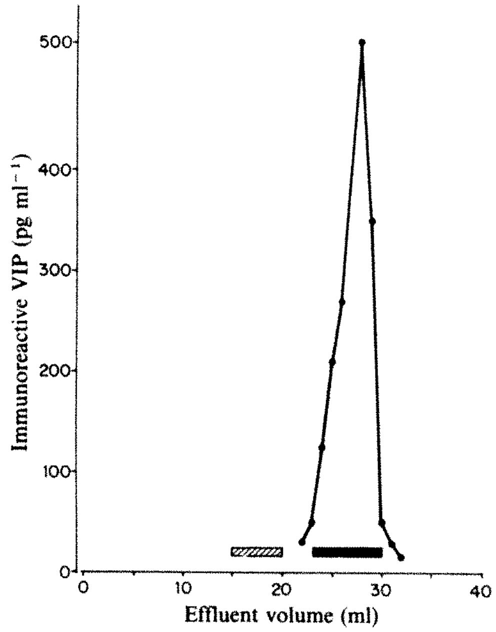

Fig. 2 Chromatogram of VIP immunoreactivity in an extract of sciatic nerves (402 mg) on a column of Sephadex G-25 Fine (0.9 × 60 cm). The nerves had been homogenised in 25 ml 0.2 M acetic acid, the homogenate centrifuged, and the supernate lyophilised and reconstituted in 0.5 ml of 0.2 M acetic acid. The column was calibrated with ^{125}I-labelled porcine VIP (solid bar) and ^{125}I-labelled albumin (shaded bar). Eluant, 0.2 M acetic acid; flow rate, 12 ml h^{-1}; fraction vol., 1 ml.

the second ligature were homogenised and assayed for VIP content.

In the control (unligated) nerve, VIP content was uniform in all segments examined and averaged 32 ± 5 pg per segment. At all intervals after nerve constriction, the concentration of immunoreactive VIP increased significantly ($P < 0.025$) in the segment just proximal to the ligature. The peptide levels increased with the duration of constriction (Fig. 1), reaching 17 and 46 times the control value after 24 and 72 h, respectively. Accumulation of the peptide occurred only in the segments proximal to the constriction (Table 1), and peptide levels decreased abruptly above the first segment (0–0.5 cm). VIP content also remained low in the segment below a second constriction (14 pg), and in the segment between two ligatures (24 pg).

On chromatography of extracts of sciatic nerves on a column of Sephadex G-25 Fine, immunoreactive VIP was eluted in one peak. The location of this peak was identical for ligated (12–72 h) and unligated nerves, and coincided with the peak of ^{125}I-labelled VIP chromatographed in the same conditions (Fig. 2). These chromatographic data suggest the presence of one molecular species of the peptide in ligated or unligated sciatic nerves.

The findings indicate axonal transport of VIP along the sciatic nerve predominantly in a proximal-to-distal direction. The absence of a concentration gradient of VIP along the contralateral nerve or in the constricted nerve more proximal to the segment immediately above the ligature suggests that the peptide is not formed *in loco*, but is probably synthesised in neuronal cell bodies and transported along the axon toward the nerve endings.

Table 1 Concentration of immunoreactive VIP in 0.5-cm segments of sciatic nerve proximal to constriction for 24 h

Segment	VIP (pg per nerve segment)
0–0.5	527 ± 117 (10)
0.5–1.0	65 ± 10 (10)
1.0–1.5	102 ± 8 (5)
1.5–2.0	103 ± 6 (5)

VIP values are means ± s.e.m. Number of determinations are in parentheses.

The rate of axonal transport of VIP (in mm h^{-1}) could be computed as: $(C - C_c/T)/(C_c/S)$, where C and C_c are the concentrations of the peptide above a constriction and in the contralateral nerve, respectively, T is the duration of constriction (in h), and S is the length of the nerve segment (in mm). From the data after 24 or 72 h constriction, the velocity of axonal transport of VIP was 3.3 mm h^{-1}. This velocity is typical of the relatively fast axoplasmic transport rates reported for cholinesterase in frog sciatic nerve[17], for certain polypeptides synthesised in rabbit retinal ganglion cells[18] and for other systems[19].

Axonal transport of peptides has previously been documented for oxytocin and vasopressin in the hypothalamo–neurohypophysial system[20–22]. Our results now show that neuronal peptides can travel by axonal transport in peripheral nerves as well. The demonstration of axoplasmic flow of VIP supports the notion that this peptide may function as a neurotransmitter or neuromodulator in peripheral nerves.

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GABA of CNS origin in the rat anterior pituitary inhibits prolactin secretion

G. Racagni*, J. A. Apud*, V. Locatelli†, D. Cocchi†, G. Nistico'‡, R. M. di Giorgio‡ & E. E. Müller§

* Institute of Pharmacology and Pharmacognosy, and † Department of Pharmacology, University of Milan, Italy
‡ Institutes of Pharmacology and Biochemistry, University of Messina, Italy
§ Institute of Pharmacology and Pharmacognosy, University of Cagliari, Italy

Hypothalamic hormones synthesised in nuclei within the medial basal hypothalamus (MBH) and extrahypothalamic regions and released from nerve endings at the level of the median eminence (ME) into the hypophysial portal capillary system control anterior pituitary (AP) function[1]. The median eminence is a key area for neuroendocrine regulation as it receives projections of neurotransmitter pathways which modulate functions such as

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synthesis and release of hypothalamic hormones[2]. In addition, transmitters released from the ME into the stalk portal blood may act directly at the AP level to control hormone secretion—this has been demonstrated for dopamine (DA) with respect to prolactin (PRL) secretion[3] and it has been suggested that DA itself may represent the so far elusive PRL-inhibiting factor (PIF)[2,4,5]. Recently, evidence has been presented which suggests that γ-aminobutyric acid (GABA) may also have an important inhibitory role in regulating PRL secretion in the rat. Both GABA and muscimol, a powerful agonist at GABA receptors[6], inhibited PRL secretion when injected into urethane anaesthetised female[7] or freely moving male rats[8]. In view of the poor penetrability of the blood-brain barrier (BBB) by both amino acids[9] and the recently described specific GABA receptor sites in rat AP[10], the above data support the notion that GABA may act directly at the level of the AP to inhibit PRL secretion. This proposition is reinforced by the demonstration that both GABA[11] and muscimol[12] inhibit PRL secretion from rat isolated APs, an effect which is blocked by the antagonist picrotoxin. We report here that GABA, derived from the central nervous sytem (CNS), is present in the rat anterior pituitary, and we provide evidence that GABA has a functional role in the control of PRL secretion.

Male Sprague–Dawley rats (150–200 g) were killed by microwave radiation and their GABA content in the anterior and posterior pituitary lobes was determined by a mass-fragmentographic technique[13] and compared with DA titres in the same tissues.

Table 1 shows that GABA is present in both the anterior and posterior lobes of the pituitary, the concentration of the amino acid being 7–8 times greater in the latter than the former tissue. DA shows a similar pattern of distribution in the pituitary, although absolute levels of the amine in both tissues are consistently lower than those of the amino acid.

Endogenous GABA in the brain is synthesised almost exclusively by decarboxylation of glutamic acid by glutamic acid decarboxylase (GAD)[14] and GABA is degraded by a transamination catalysed by α-ketoglutarate-GABA transaminase (GABA-T)[15]. It is evident from Table 1 that the AP, despite the presence of GABA, does not contain the GABA biosynthetic machinery; no GAD activity was detectable at this level whereas GAD activity was present in the hypothalamus and, although at low levels, also in the posterior lobe. This finding is analogous to the finding that DA is present in the AP even though its biosynthetic enzyme, tyrosine hydroxylase cannot be detected[16]. A further similarity between GABA and DA at AP level is the fact that for both the amino acid transmitter and the amine, the physiological catabolic enzymes, that is, GABA-T (Table 1) and monoamine oxidase[16] respectively, are present in the glandular tissue. GABA-T activity was also detected in the hypothalamus at concentrations similar to those found in the AP (Table 1).

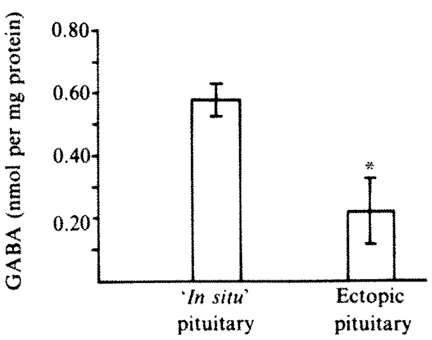

Fig. 1 GABA concentration in the ectopic or in *situ* anterior pituitary gland. Female Sprague–Dawley rats (100–130 g) were hypophysectomised by the transauricular route[36] and 15 days later received implants of one anterior pituitary, obtained from an intact female littermate, under the kidney capsule. After transplantation and before the experiments, animals were weighed daily; only animals which showed a constant body weight increase throughout this period were included in these studies. GABA concentrations were determined in the ectopic gland 10 days after transplantation (ref. 13). Age- and weight-matched female rats served as controls. Significance of differences between groups was calculated by Student's *t*-test. Values represent the mean + s.e.m. of six determinations. $^*P < 0.01$.

Taken together these findings argue against the possibility of local formation of GABA in the AP and suggest instead that the gland may merely be a tissue for storage and catabolism of GABA manufactured elsewhere.

Ethanolamine-*O*-sulphate (EOS), a structurally related analogue of GABA, is a potent, specific, competitive and irreversible catalytic inhibitor of GABA-T *in vitro* and *in vivo*[17]. Experiments were performed to determine whether GABA stored in the AP originated in the CNS. Table 2 shows that EOS injected intracerebroventricularly (i.v.t.) at 2 mg per kg bodyweight into the lateral ventricle of freely behaving unanaesthetised rats induced an increase in GABA content not only in the hypothalamus but also in the anterior and posterior pituitary

Table 1 GABA concentrations and GAD and GABA-T activities in rat pituitary lobes and hypothalamus

Tissue	GABA (nmol per mg protein)	GAD (pmol CO_2 per mg protein per h)	GABA-T (nmol glutamate per mg protein per h)	Dopamine (pmol per mg protein)
Anterior lobe	0.76 ± 0.07	None	324.6 ± 21.1	1.25 ± 0.13
Posterior lobe	5.53 ± 0.60	0.574 ± 0.017	245.2 ± 21.2	53.71 ± 10.01
Hypothalamus	40.11 ± 3.81	6,320 ± 450	224.7 ± 17.0	66.63 ± 3.22

To avoid variability due to circadian fluctuations of GABA content in the hypothalamus[28], these and the following experiments (Tables 2, 3 and Fig. 1) were conducted in the early afternoon. The brain and pituitaries from microwave-radiated (2.0 kW; 2.45 GH; 75 W cm^{-2})[29] male Sprague–Dawley rats were rapidly removed, and the hypothalamus was dissected from the brain according to the procedure described in ref. 30. The weight of an hypothalamic fragment was about 20–25 mg. Pituitaries once removed were washed in cold saline and posterior and intermediate lobes were entirely removed from the isthmus to assure separate analysis of pure anterior and posterior (neural and intermediate) lobes. The mean weight of an anterior or posterior pituitary expressed as mg protein was about 0.5 or 0.1, respectively. For GABA determination, excised hypothalami and pituitary lobes were immediately frozen in dry ice, and then homogenised in 0.1 M formic acid (1 ml for hypothalamus and 200 μl for pituitary lobes) containing the internal standard 4-aminovaleric acid (2 μmol ml^{-1}). The homogenates were centrifuged at 15,000*g* for 20 min and an aliquot of the supernatant was transferred and evaporated under reduced pressure. A mixture of SIL PREP and BSTFA (3 : 1) (Applied Science, Lab. Inc. State College, Bellefonte, Pen.) was added. After incubation for 2 h at room temperature, an aliquot of the reaction mixture was injected into a gas chromatograph–mass spectrometer (Finnigan 3000 E), focused on *m/e* 304 and 174 for GABA-TMS derivative and *m/e* 304 for the internal standard according to Cattabeni *et al.*[13]. DA was also analysed with a mass fragmentographic technique, as described by Cattabeni *et al.*[31]. GAD was assayed by measuring the evolution of $^{14}CO_2$ from L[1-^{14}C]glutamic acid in anaerobic conditions[32]. The $^{14}CO_2$ formed was absorbed in hyamine solution and counted in a liquid scintillation counter. In a typical assay, the incubation vessel contained 0.1 ml of 0.208 M glutamic acid (0.74 μCi L[1-^{14}C]glutamate) in 0.1 M K_3PO_4 buffer containing 0.2 mM pyridoxal phosphate (PLP) *p*H 7.2. The reaction was started by injecting 1 ml enzyme solution in 50 mM K_3PO4 buffer, *p*H 7.2, containing 0.2 mM PP and 1 mM AET (standard buffer) into the incubation vessel. The incubation was performed in a Dubnoff metabolic incubator for 30 min at 37 °C at approximately 150 r.p.m. and was terminated by injecting 0.1 ml of 4 M H_2SO_4 into the reaction mixture. To ensure complete release of CO_2 and absorption in the hyamine base, vessels were incubated for additional 60 min. GABA-T activity was assayed according to Macaione[33] in 100 μl of the reaction mixture containing: GABA, 5 μM; α-ketoglutarate, 5 μM; PLP, 0.4 μM; 0.1 M phosphate buffer, *p*H 7.4 and homogenate (w/v1 : 10). After 1 h incubation at 37 °C, the reaction was stopped by adding 0.4 ml of absolute ethanol. Controls were carried out by adding homogenate after ethanol. GABA content in the homogenate and glutamic acid formed by GABA-T activity were quantitated by means of an amino acid analyser (Carlo Erba). Proteins were determined by the procedure of Lowry *et al.*[34]. For GABA and DA, values represent the mean ± s.e.m. of at least five determinations; for GAD in the pituitary lobes and hypothalamus, values represent the mean ± s.e.m. of five determinations obtained from a pool of 10 glands or 4 hypothalamic fragments, respectively; for GABA-T in the AP and hypothalamus, values represent the mean ± s.e.m. of 6 determinations obtained from 12 tissue samples.

lobes. In the AP the increase in GABA concentration was time related. EOS does not cross the BBB[17] so it is unlikely that these changes are caused by an effect of the drug on GABA catabolism in the systemic circulation. Supporting this view is the observation that intravenous injection of EOS, at a dose 25 times greater than the i.v.t.-injected dose, that is, 50 mg per kg, did not alter GABA concentrations either in the hypothalamus or the pituitary gland (Table 2).

If, as implied by experiments with EOS, the main source of GABA in the AP is via transport of the amino acid from the CNS to the pituitary, location of the pituitary in a site far removed from the sella turcica should result in a lowering of the pituitary concentration of the amino acid. Figure 1 shows that in a gland transplanted under the kidney capsule, the GABA concentration is three times lower than in the pituitary gland *in situ*.

Table 2 GABA levels in rat pituitary gland and hypothalamus after central or systemic administration of ethanolamine-*O*-sulphate (EOS)

	GABA (nmol per mg protein)		
Treatment	Anterior lobe	Posterior lobe	Hypothalamus
Central route			
Saline 10 μl per rat	0.64 ± 0.07	6.05 ± 1.26	48.21 ± 5.40
EOS (2 h)	1.27 ± 0.35*	13.18 ± 3.31*	120.65 ± 15.61*
EOS (4 h)	3.17 ± 0.87*†	19.32 ± 4.97*	150.41 ± 24.50*
Systemic route			
Saline 0.5 ml per rat	0.71 ± 0.18	4.28 ± 1.10	40.11 ± 3.81
EOS (2 h)	0.59 ± 0.20	4.32 ± 1.67	41.33 ± 4.03
EOS (4 h)	0.60 ± 0.17	5.47 ± 1.91	39.67 ± 3.63

In the central experiments, male Sprague–Dawley rats (200–220 g) had a cannula implanted into the lateral ventricle of the brain[35] while under barbiturate anaesthesia. After allowing 2–3 days for recovery, EOS (2 mg per kg in 10 μl saline) was slowly infused through the implanted cannula. When EOS was injected by systemic route, an intravenous (i.v.) dose of 50 mg per kg was used. Animals were killed by microwave radiations 2 and 4 h after EOS administration. Tissues were dissected and assayed for GABA as described in Table 1 legend (ref. 13). Values represent the mean ± s.e.m. of 10 determinations (pooled data of two separate experiments). Significance of difference between groups was calculated by Student's *t*-test.

*$P < 0.01$ versus saline-injected rats;

†$P < 0.01$ versus EOS-injected rats (2 h).

Proof that GABA, probably derived from the CNS and transported into stalk blood to the AP, exerts a functional role in the control of PRL secretion is provided by the experiments summarised in Tables 3 and 4. Table 3 shows that, concomitant with an increase in hypothalamic content of GABA by i.v.t.-injected EOS, there was a clear-cut decrease in plasma concentration of PRL; in contrast, EOS administered systemically affected neither hypothalamic GABA concentrations nor plasma PRL titres. The case for a causal relationship between changes induced in the hypothalamic and pituitary concentrations of GABA and changes in PRL release is strengthened by the observation that they occurred independently of alterations in DA function. In fact, i.v.t. administration of 2 mg per kg EOS, which induced clear-cut rises in GABA concentrations in the hypothalamus and the pituitary, did not affect the DA level in the AP, which is a reliable marker of tubero-infundibular DA function[18] (2.5 ± 1.03 and 2.9 ± 0.35 pmol per mg protein in saline- and EOS-injected rats, respectively). Furthermore, central administration of EOS antagonised the PRL-releasing effect evoked by blockade of DA receptors with domperidone, a drug which, being unable to cross the BBB[19,20], acts almost exclusively at the AP level (Table 4).

Although GABA is a neurotransmitter involved in the control of AP hormone secretion[2], the mechanism(s) underlying its neuroendocrine effects have yet to be clearly established. For instance conflicting data have been presented, indicating both a stimulatory[21–23] and, an inhibitory[7,8,12,24] role for GABA in the regulation of PRL secretion. The data presented here show GABA to be present in the AP gland and offer a plausible explanation for the mechanism underlying the PRL-lowering effect of the amino acid.

Table 3 Prolactin concentrations in the plasma and GABA content in the hypothalamus after i.v.t. or i.v. administration of EOS

	Prolactin (ng ml^{-1})		GABA (nmol per mg protein)	
Treatment	EOS (2 mg per kg) central route	EOS (50 mg per kg) systemic route	EOS (2 mg per kg) central route	EOS (50 mg per kg) systemic route
Saline	20.5 ± 5.5	14.6 ± 3.5	40.01 ± 2.13	45.50 ± 2.60
EOS (2 h)	3.0 ± 0.9*	17.1 ± 1.9	138.99 ± 13.60*	40.20 ± 4.09
EOS (4 h)	9.5 ± 4.2*	15.4 ± 2.4	161.18 ± 24.11*	38.90 ± 3.90

In these experiments central and systemic administration of EOS were performed following the schedule described in the legend to Table 2. Immediately before and 2 and 4 h after EOS administration, male rats were sampled by the retroorbital venous technique[37] for PRL determination in the plasma. PRL was determined by a double antibody radioimmunoassay (RIA), using the method of Niswender *et al.*[38]. All results were expressed in ng ml^{-1} in terms of the NIH standard rat PRL-RP-1, whose potency is 11.0 IU mg^{-1}. The sensitivity of the PRL assay is 1.0 ng ml^{-1}; intra-assay variation was 5% while repeated assay of a reference plasma showed an intra-assay variation of 6%. To avoid possible intra-assay variations, all samples were assayed in a single RIA. The hypothalamus was dissected and GABA was assayed as described in Table 1 legend. Values represent the mean ± s.e.m. of at least five determinations.

*$P < 0.01$ versus saline-injected rats by Student's *t*-test.

Detection of GABA at AP level extends the list of neurotransmitters which have been identified in this glandular tissue[16]. In the absence of a synthetic pathway in the AP, the function of GABA-T at the AP level would be mainly to catabolise GABA released from CNS centres and then transported through the hypophysial portal circulation. The high GABA-T activity found in the AP could readily serve this function. However, in view of the maintenance of GABA titres, although low, in the ectopic gland, we cannot exclude the possibility that a fraction of the GABA measured in the AP gland *in situ* may derive from the systemic circulation.

Table 4 Effect of i.v.t. administration of EOS on the PRL rise induced by blockade of pituitary DA receptors with domperidone

	Plasma prolactin (ng ml^{-1})		
Treatment	Δt_{30}	Δt_{60}	Δt_{90}
Saline + DOM (8)	23.0 ± 5.6	25.8 ± 6.2	26.8 ± 5.7
EOS + DOM (7)	13.1 ± 3.4	13.6 ± 5.0	10.0 ± 2.3*

Sprague–Dawley male rats (200–220 g) were implanted via the lateral ventricle of the brain[32] and prepared with chronic indwelling jugular cannulae inserted into the right atrium[39]. After 2–3 days for recovery, they were given EOS (2 mg per kg in 10 μl saline) or saline injection and 3 h later received an i.v. injection of domperidone (DOM, Motilium, Janssen) 0.01 mg per kg, and were sampled from the jugular cannula for RIA determination of plasma PRL. Values refer to incremental changes (Δ) in plasma PRL (mean ± s.e.m.) recorded at various times after DOM administration (30, 60 and 90 min). Numbers of rats are indicated in parentheses. Baseline PRL levels were 7.8 ± 1.5 (mean ± s.e.m.) and 3.8 ± 0.7 ng ml^{-1} ($P < 0.05$) in saline + DOM and EOS + DOM-injected rats, respectively. Calculation of the areas subtended by PRL profiles revealed a significant difference between saline + DOM and EOS + DOM treated groups (181 ± 19 mean ± s.e.m. versus 122 ± 16, respectively, $P < 0.05$ by Student's *t*-test).

*$P < 0.05$ versus saline + DOM injected rats by Student's *t*-test.

The organisation of GABAergic pathways in the hypothalamus provides neuroanatomical evidence for an action of GABA exerted directly at the AP level. In addition to hypothalamic GABAergic fibres projecting to the MBH, there is evidence for the existence of a medial basal hypothalamic (tubero-infundibular) pathway which projects to the ME[25]. Experiments reported here involving i.v.t.-injected EOS suggest that the increase in GABA content at the AP level was the consequence of inhibition of GABA-T in this pathway with ensuing GABA loading in the stalk circulation. The time-related rise in AP GABA concentration after EOS strongly suggests this mechanism. GABA once released into the portal circulation would be competent to control in an inhibitory fashion PRL secretion from the AP, acting at the level of specific GABA receptor sites[10]. Since EOS, in contrast to muscimol[6], is unable to affect GABA receptor sites directly[17], the above is the more plausible explanation for the PRL-lowering effect of the centrally administered compound. Our data suggest that the inhibitory effect of GABA on plasma PRL occurs independently of DA changes.

The finding of GABA in the posterior lobe of the pituitary, and the increases in GABA titres after blockade of GABA-T confirm previous data[26] and may reflect a functional role for the amino acid at this level. A functional role for DA present in the neuro-intermediate lobe has been recently postulated[27].

The findings reported here underline the functional analogy between the anatomical and biochemical organisation of GABA and DA at ME–AP level, and suggest an important role for the amino acid in the control of PRL secretion.

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Dopamine receptors in the retina may all be linked to adenylate cyclase

Keith J. Watling, John E. Dowling & Leslie L. Iversen

MRC Neurochemical Pharmacology Unit, Department of
Pharmacology, Medical School, Hills Road, Cambridge, UK

Dopamine seems to be the principal catecholamine in the vertebrate retina, where it is found in two types of neurone[1]. In most species, there is a subclass of amacrine cell which makes synapses on other amacrine cells in the inner plexiform layer[2]. In teleost fish and New World monkeys, the dopaminergic neurones are interplexiform cells which make synapses in both plexiform layers on horizontal, bipolar and amacrine cells[3]. In other dopamine-rich areas of the central nervous system, there is evidence for multiple dopamine receptor types[4–7], and two sub-types have been suggested[8]. D-1 receptors are associated with an adenylate cyclase system whereas D-2 receptors are not. D-2 receptors are characterised by a high affinity for the butyrophenones haloperidol[9] and spiperone[10], but these ligands also apparently bind to D-1 receptors[11]. The butyrophenone domperidone also binds to central dopamine receptors[12], but it is a very weak antagonist of dopamine-sensitive adenylate cyclase[13] (Table 1), suggesting that it may bind only to D-2 receptors. In the retina of many species, a dopamine-sensitive adenylate cyclase has been demonstrated[14,15], indicating the presence of D-1 receptors. We report here that there is no significant receptor-specific binding of ^{3}H-domperidone to retinal membranes, thus suggesting the absence of D-2 receptors in this tissue.

The dopamine-sensitive adenylate cyclase in the retina seems to be identical to that found elsewhere in the brain. Figure 1 shows, for example, adenylate cyclase activity in homogenates of the retina and corpus striatum of the guinea pig prepared in similar conditions. In both homogenates, dopamine elicits a dose-dependent stimulation of cyclic AMP formation, with maximal stimulation occurring at concentrations of 0.1–1 mM, and half-maximal stimulation with approximately 10μM dopamine. When expressed in terms of cyclic AMP produced above baseline levels, an average maximal response of 38.3 ± 6.7 pmol cyclic AMP per mg protein per min was obtained in four striatal homogenates, whereas a value of 47.3 ± 4.6 pmol per mg protein per min was obtained in seven retinal homogenates. These results indicate that there are substantial

Table 1 Inhibition of dopamine-stimulated adenylate cyclase in homogenates of guinea pig and carp retinae and rat striatum

	K_i values (M)		
Drug	Guinea pig retina	Carp retina	Rat striatum
Fluphenazine	3.9×10^{-9}	3.0×10^{-9}	4.3×10^{-9}
α-Flupenthixol	4.8×10^{-9}	5.1×10^{-9}	1.0×10^{-9}
(+)Butaclamol	2.4×10^{-8}	2.5×10^{-8}	8.8×10^{-9}
Chlorpromazine	4.5×10^{-8}	7.3×10^{-8}	4.8×10^{-8}
Haloperidol	9.0×10^{-8}	9.4×10^{-8}	9.1×10^{-8}
(−)Butaclamol	$>1\times10^{-6}$	$>1\times10^{-6}$	$>1\times10^{-6}$
Domperidone	$>1\times10^{-6}$	$>1\times10^{-6}$	$>1\times10^{-6}$

The effect of each drug on dopamine-stimulated adenylate cyclase activity in homogenates of carp and guinea pig retina was examined at six concentrations in the range 0.01–100 μM. Data are the results from two or three separate experiments involving quadruplicate determinations and s.e.m. values of < 10% of the mean. Drugs were added immediately before dopamine. K_i Values for each drug were calculated from the equation $IC_{50} = K_i(1 + S/K_m)$, where IC_{50} is the concentration of drug required to produce 50% inhibition of the maximum dopamine-induced response, S is the concentration of dopamine used (100 μM) and K_m is the concentration of dopamine producing half-maximal stimulation of adenylate cyclase (guinea pig, 10 μM: carp, 1 μM); $>1\times10^{-6}$ M = less than 50% inhibition at 100 μM. Competitive inhibition has been assumed for all compounds tested. K_i values for rat striatum are taken from previous reports (refs 5, 13, 16).

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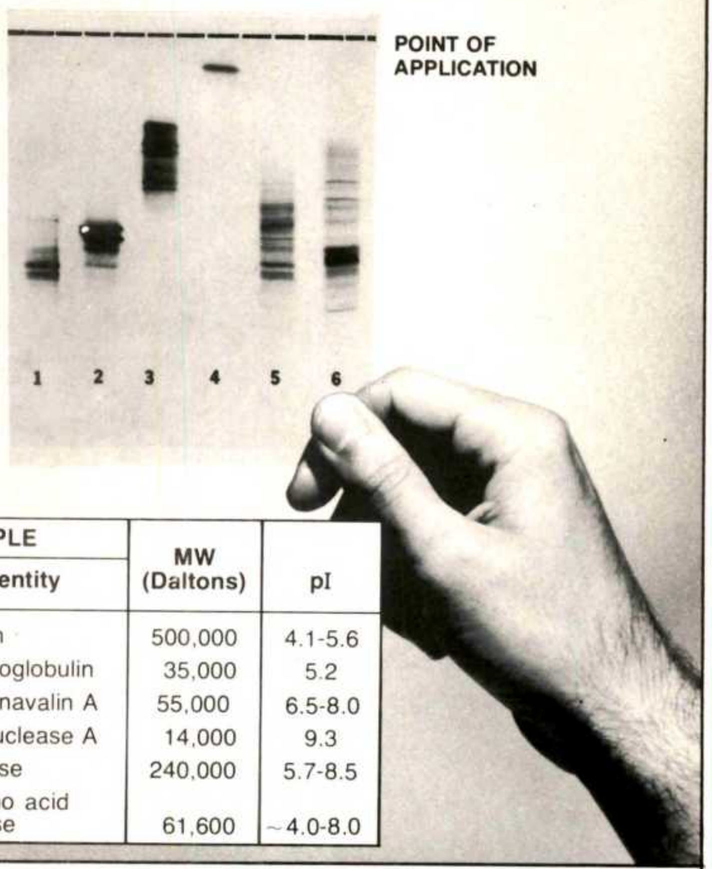

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4	Ribonuclease A	14,000	9.3
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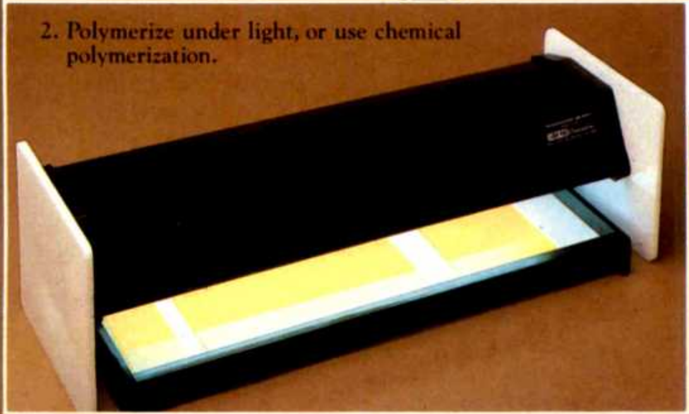
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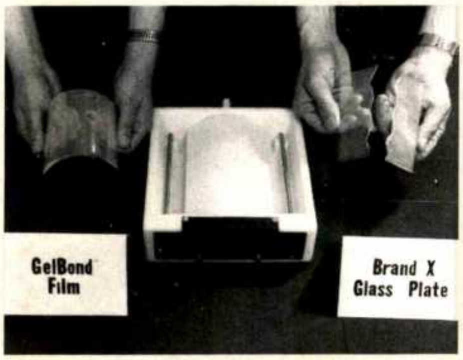

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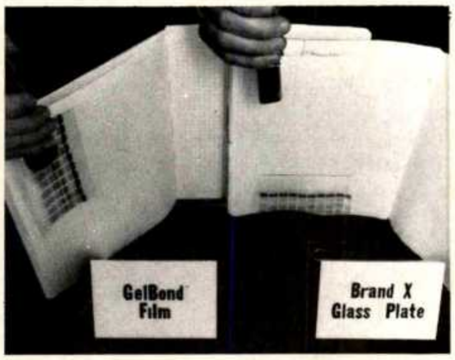

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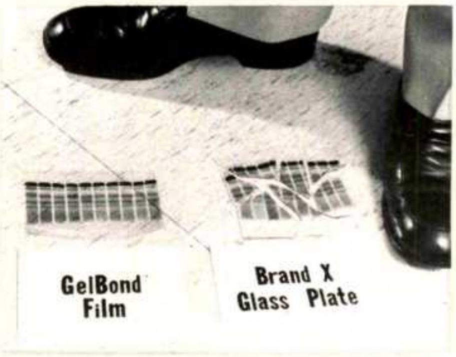

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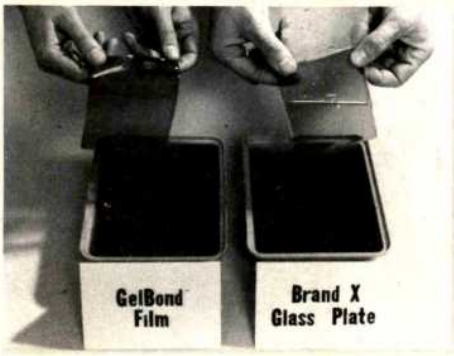

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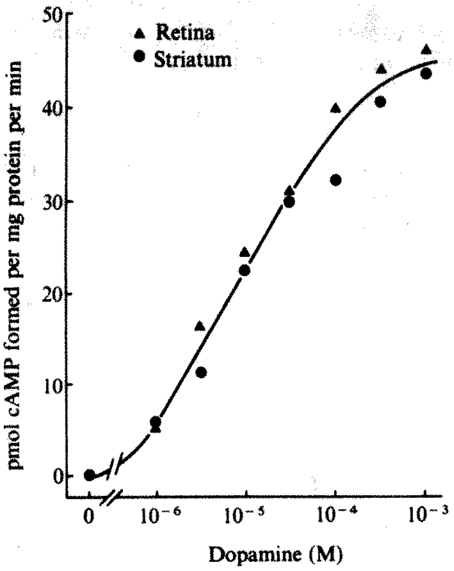

Fig. 1 Effect of dopamine on cyclic AMP production in homogenates of guines pig retina and striatum. Results are expressed as pmol cyclic AMP per mg protein per min produced above baseline levels. Each point is the mean of three separate experiments involving quadruplicate determinations. s.e.m. values were less than 20% of the mean. Adenylate cyclase activity was assayed using the method of Kebabian *et al.*[17] as follows. Retinae and striata were removed from guinea pigs (250–300 g) and separately homogenised in 2 mM Tris-maleate buffer containing 2 mM EGTA. Retinae were homogenised using a ratio of 1 retina to 500 μl buffer, and striata using a ratio of 1 mg wet weight striata to 25 μl buffer. Aliquots (20 μl) of this homogenate were added to assay tubes containing 130 μl of 80 mM Tris-maleate buffer (*p*H 7.4), 2 mM $MgSO_4$, 10 mM theophylline and 0.2 mM EGTA plus dopamine as indicated. The reaction in each tube was initiated by the addition of 50 μl ATP to a final concentration of 1 mM. The tubes were incubated with shaking at 30°C for 3 or 5 min and transferred to a boiling water bath for 3 min followed by centrifugation. Duplicate 10 μl samples of the final supernatant were assayed for cyclic AMP content by the method of Brown *et al.*[18]. Baseline cyclic AMP levels in four striatal homogenates were 70.6 ± 12.8 pmol cyclic AMP per mg protein per min and 19.4 ± 2.9 pmol cyclic AMP per mg protein per min in seven retinal homogenates. Protein contents were determined according to the method of Lowry *et al.*[19] using bovine serum albumin as a standard.

amounts of dopamine-sensitive adenylate cyclase, and presumably D-1 receptors, in both guinea pig retina and striatum.

Furthermore, in guinea pig retinal homogenates, neither the α-adrenoreceptor agonist phenylephrine nor the β-adrenoreceptor agonist isoprenaline produce any significant stimulation of adenylate cyclase at concentrations up to 100 μM, indicating that the catecholamine receptors linked to adenylate cyclase in the retina are specific for dopamine. In addition, various dopaminergic antagonists, including fluphenazine, α-flupenthixol, chlorpromazine and haloperidol, inhibit the retinal dopamine-sensitive adenylate cyclase with K_i values similar to those previously obtained in striatal homogenates[5] (Table 1). The dopamine-sensitive adenylate cyclase in retinal homogenates is also antagonised by the neuroleptic agent (+)-butaclamol, whereas (−)butaclamol is essentially inactive. This stereospecificity is also similar to that observed in striatal homogenates[5]. Finally, in agreement with results obtained in homogenates of rat striatum[13], domperidone shows no significant antagonism of the retinal dopamine-sensitive adenylate cyclase at concentrations up to 100 μM.

Significant binding of ^{3}H-domperidone can be demonstrated readily in guinea pig striatal homogenates (Fig. 2), and 85–90% of this binding is displaced by non-radioactive dopamine at a concentration of 1 mM, with 50% displacement of specific binding at 1 μM dopamine. These data indicate that there are many dopamine-specific binding sites for domperidone in the guinea pig striatum. In contrast, comparatively little ^{3}H-domperidone binding is detectable in similarly prepared homogenates of guinea pig retinae, and dopamine at concentrations up to 10 μM fails to displace any of the bound drug. A small amount of displacement seems to occur with dopamine concentrations of 100 μM and 1 μM, but this effect is not statistically significant.

The amount of non-displaceable ^{3}H-domperidone binding observed in retinal homogenates is comparable to the level of nonspecific binding remaining in striatal homogenates incubated with 1 mM dopamine, and probably represents binding of the ligand to non-receptor sites. In one homogenate of the rabbit retina there was a comparable small amount of ^{3}H-domperidone binding (2.5 fmol per mg wet weight tissue) which was also not affected by dopamine at concentrations up to 1 mM.

We have also studied the dopamine-sensitive adenylate cyclase and ^{3}H-domperidone binding in the carp retina, whose dopaminergic neurones are of the interplexiform type[3]. As in the guinea pig, a prominent dopamine-sensitive adenylate cyclase is present in this retina. This cyclase also seems to be identical to those in other retinae and other brain regions in terms of its specificity for dopamine, sensitivity to various neuroleptic drugs but insensitivity to domperidone (Table 1). As in the other retinae examined, no dopamine-displaceable ^{3}H-domperidone binding was observed in homogenates of the carp retina in five experiments. However, in carp, the amount of non-displaceable binding was approximately twice that found in the guinea pig (4.4 fmol, per mg wet weight compared with 2.8 fmol per mg wet weight).

In preliminary experiments, we have examined the binding of another butyrophenone derivative, ^{3}H-spiperone, to guinea pig and carp retinal homogenates. This ligand apparently binds to both D-1 and D-2 receptors in striatal homogenates[11]. In

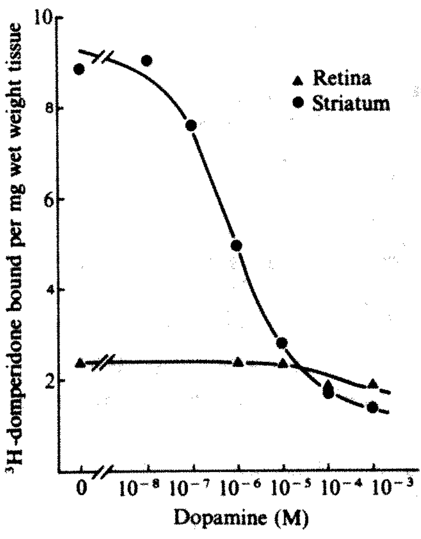

Fig. 2 Effect of dopamine on the binding of ^{3}H-domperidone to guinea pig striatal and retinal homogenates. Binding of ^{3}H-domperidone (corrected for filter blanks) to guinea pig striatal or retinal membranes, in the absence or presence of various concentrations of dopamine, is expressed as fmol bound per mg wet weight tissue. Each point represents the mean of four experiments involving triplicate determinations. s.e.m. values were less than 15% and 30% of the mean in the striatum and retina, respectively. Membrane preparations were prepared as previously described[10,20,21]. Incubation tubes containing 50 μl ^{3}H-domperidone (Janssen, specific activity 10 Ci $mmol^{-1}$) to give a final concentration of 1 nM, 50 μl dopamine (final concentration as indicated) or distilled water and 900 μl homogenate (equivalent to ~5 mg wet weight of original tissue for striatum and 7–10 mg wet weight of original tissue for retina) were incubated for 20 min at 30°C. Membranes were then collected by filtration, washed, and the bound radioactivity determined[10,20,21]. Total binding of ^{3}H-domperidone was 8.87 ± 0.16 and 2.38 ± 0.38 fmol per mg wet weight tissue in striatum and retina, respectively. In the presence of 1 mM dopamine, this binding was reduced to 1.38 ± 0.11 and 1.86 ± 0.39 fmol per mg mg wet weight tissue.

agreement with this, we have observed a significant amount of dopamine-displaceable ^{3}H-spiperone binding in both these retinae.

In conclusion, our results suggest that the retina may represent an area of the vertebrate central nervous system which contains a pure population of D-1 receptors; that is, all linked to adenylate cyclase. This situation apparently holds regardless of the type of dopaminergic neurone present in the retina. Thus, whether the dopaminergic neurones are a class of amacrine cell, as in the guinea pig or rabbit, or interplexiform cells, as in the carp, no D-2 dopamine receptors seem to be present. These results further imply that all neurones postsynaptic to the retinal dopaminergic cells, namely amacrine cells in the guinea pig and rabbit, and bipolar, horizontal and amacrine cells in the carp, possess a dopamine-sensitive adenylate cyclase.

We thank John Horne for carrying out several of the experiments on the guinea pig retinal adenylate cyclase and Dr P. Laduron (Janssen) for providing the ^{3}H-domperidone. K.J.W. is a Fellow of the Parkinson's Disease Society, London. J.E.D. was on leave from Harvard University and a Simon A. Guggenheim Fellow, 1978–79.

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Combined effects of adrenaline and insulin on active electrogenic Na^+–K^+ transport in rat soleus muscle

J. A. Flatman & T. Clausen

Institute of Physiology, University of Aarhus, 8000 Aarhus C, Denmark

Both β_2-adrenoreceptor stimulants (such as adrenaline and salbutamol) and insulin can increase active Na^+–K^+ transport[1–6] and hyperpolarise skeletal muscle cells[1,2,7–9]. Thus, adrenaline and insulin, which are otherwise antagonistic regulators of several metabolic processes, have one action in common, namely, stimulation of active ion translocation. This is especially interesting as cyclic AMP stimulates Na^+–K^+ transport[2], whereas a lowering of the cytoplasmic concentration of cyclic AMP has been proposed as an early signal in the action of insulin[10,11]. Here we report the results of experiments in which the active Na^+–K^+ transport and membrane potential (E_M) of rat soleus muscles were studied during the action of supramaximal doses of insulin and β_2-adrenoreceptor stimulants, alone and in combination. We conclude that the stimulant action of insulin on active electrogenic Na^+–K^+ transport is unlikely to be evoked by a lowering of the intracellular concentration of cyclic AMP.

All experiments were carried out using the rat soleus muscle. The techniques for measuring Na^+–K^+ transport and E_M have been described in detail previously[2,6,12]. For the measurements of the content and transport of Na^+ and K^+, muscles obtained from rats weighing 60–70 g were used. It was earlier demonstrated that there is no significant difference between the E_M of muscles from small (60–70 g) and large (200–400 g) rats[2]. As large fibres allow more stable recordings, the major part of the present measurements *in vitro* and *in vivo* were carried out using animals weighing 200–400 g.

In vivo recordings of E_M were made using pentobarbitone-anaesthetised rats weighing 300–400 g. In these experiments, the dorsal surface of the right soleus muscle was exposed at the bottom of a 'pool' formed by lifting the edges of the skin incision. The muscle surface was covered by a thin layer of standard Krebs–Ringer bicarbonate buffer on which a light paraffin oil was layered. The temperature of the pool and the body of the animal was maintained at 37 °C using a thermostated heating blanket. Venous blood samples were taken through a catheter in the left superior vena cava and drugs infused through a catheter in the left femoral vein. A Ag/AgCl reference electrode was buried in the left thigh muscles (the details of this technique are described elsewhere[13]).

Supramaximal concentrations of either adrenaline (10^{-5} M) or insulin (100 mU ml^{-1}) stimulated ^{22}Na efflux and ^{42}K influx (Table 1), and increased E_M within approximately the same interval of time (Table 2). These large doses were used to ensure that maximal stimulation was evoked by each agent alone. The effects of insulin were somewhat less pronounced and slower in onset than those of adrenaline. Ouabain (10^{-3} M) blocked the hyperpolarising effect of both hormones (Table 2), indicating that this is related to a stimulation of active Na^+–K^+ transport. The effects of adrenaline and insulin were partially additive on Na^+–K^+ transport, whether the insulin was added before, after or simultaneously with adrenaline (as in Table 1). On the other hand, although the addition of adrenaline to muscles already exposed to insulin gave a further hyperpolarisation, this was smaller than that produced by adrenaline alone. Moreover, the hyperpolarisation produced by adrenaline was significantly reduced by the later addition of insulin. Similar results were obtained with salbutamol alone or combined with insulin (Table 2).

Also *in vivo*, salbutamol and adrenaline produced a hyperpolarisation[13,14]. This was dose dependent and accompanied by a small reduction of plasma K^+. The hyperpolarisation produced by a supramaximal salbutamol concentration *in vivo* was larger than that evoked by a similar concentration *in vitro*. Insulin, whether released in response to the intravenous (i.v.) injection of glucose (Table 3) or injected i.v., also produced a hyperpolarisation and a non-significant fall of plasma [K^+]. The i.v. injection of 0.5 IU insulin to a 250-g rat produced a hyperpolarisation of 15.2 mV (from −69.9 ± 0.6 [39 observations] to −85.1 ± 0.5 [44]) which was seen in all four fibre layers investigated. The much larger response to exogenous insulin is poss-

Table 1 Effect of insulin and adrenaline on ^{22}Na washout and ^{42}K uptake in rat soleus muscle

	^{22}Na Efflux (min^{-1})	*P*	^{42}K Influx (μmol per g per min)	*P*
Control	0.058 ± 0.002 (5)		0.54 ± 0.02 (8)	
		<0.001		<0.005
Insulin (100 mU ml^{-1})	0.081 ± 0.004 (6)		0.65 ± 0.03 (8)	
		<0.005		<0.001
Insulin (100 mU ml^{-1}) + adrenaline (10^{-5} M)	0.103 ± 0.004 (6)		0.81 ± 0.02 (8)	
		>0.10		<0.01
Adrenaline (10^{-5} M)	0.098 ± 0.004 (6)		0.71 ± 0.02 (8)	

For experimental details, see refs 2 and 6. The rate of ^{22}Na washout is given as the fraction of ^{22}Na activity lost from the tissue per min, and ^{42}K uptake as the amount of ^{42}K activity gaining access to the tissue compartment not available to ^{14}C-sucrose. The measurements were carried out within the first 10 min of exposure to the hormones, and all results are given ±s.e.m. with the number of observations in parentheses.

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Table 2 The action of insulin and adrenaline on the E_M of rat soleus muscle

Muscle	Additions	ΔE_M(mV)	*n*	*P*
(i)				
a	Insulin	+3.5±0.2	(199/5)	<0.001
	Insulin + adrenaline	+7.3±0.3	(192/5)	
b	Adrenaline	+7.4±0.4	(194/5)	<0.001
	Adrenaline + insulin	+6.0±0.3	(182/5)	
a	Insulin	+3.6±0.4	(151/4)	<0.001
	Insulin + salbutamol	+9.3±0.4	(146/4)	
b	Salbutamol	+9.1±0.4	(144/4)	<0.10
	Salbutamol + insulin	+8.1±0.4	(155/4)	
(ii)				
a	Insulin	+4.3±0.4	(171/4)	<0.001
b	Ouabain + insulin	−0.5±0.4	(172/4)	
a	Adrenaline	+5.8±0.7	(115/5)	<0.001
b	Ouabain + adrenaline	−0.9±1.0	(136/5)	

(i) The action of insulin (100 mU ml^{-1}) and adrenaline (10^{-5} M), or insulin (100 mU ml^{-1}) and salbutamol (10^{-5} M), applied individually or in combination, on the E_M of rat soleus muscle fibres (animal size 200–400 g). Paired experiments were carried out in which insulin was added either before (muscles *a*) or after (muscles *b*) the β_2-adrenoreceptor agonist (using contralateral muscles of the same rats). A series of penetrations was first made while the muscle was bathed in drug-free Krebs–Ringer bicarbonate buffer (control). A further series of penetrations was started when the maximum drug-induced hyperpolarisation had been reached, that is, about 7 min after the bath concentration of β_2-adrenoreceptor agonist was brought to 10^{-5} M (ref. 2). In the experiments with insulin, the maximum rise was only reached 15 min after the onset of exposure. Although the ion contents of the muscle as a whole continued to change after these time intervals, no further change of potential was noted. The mean (±s.e.m.) changes in E_M produced by drug addition, compared with the drug-free control E_M, are tabulated (ΔE_M). *n* Indicates the number of fibres penetrated and the number of muscles investigated.

(ii) The action of insulin (100 mU ml^{-1}) and adrenaline (6×10^{-6} M) on the E_M of rat soleus muscle fibres in the absence and presence of ouabain (10^{-3} M). Paired experiments were carried out in which insulin was added to the soleus muscles of 250-g rats individually, or after the muscles had been pretreated with ouabain (contralateral muscles). This lasted 30 min to allow time for ouabain-induced muscle fibrillations to cease. In a corresponding series carried out on 60-g rats adrenaline was added alone or after ouabain pretreatment. The penetrations were timed as in (i). The *P* values given express the significance of difference between ΔE_M values obtained with one agent alone or two agents combined. Except in the presence of ouabain, all test values were significantly different ($P < 0.001$) from the control values obtained in drug-free solution.

Table 3 Effect of i.v. administration of glucose on serum K$^+$, serum insulin and E_M of rat soleus muscle *in vivo*

	Control ±s.e.m.	10–20 min after glucose i.v. ±s.e.m.	Δ	*n*	*P*
Serum [K$^+$] (mM)	4.2±0.2	3.9±0.1	−0.3	8/4	>0.10
Serum insulin (μM ml^{-1})	20±3	179±16	+159	4/2	<0.001
E_M (mV)	−70.3±0.6	−75.8±0.6	+5.5	130/4	<0.001

Female fed rats weighing 300–400 g were anaesthetised with pentobarbitone and the dorsal surface of the right soleus muscle exposed to allow the recording of E_M. Following a control period, each animal was given an i.v. injection of D-glucose (1 mg per g body weight) and the changes in serum K$^+$, serum immunoreactive insulin and E_M were recorded. Control experiments in which mannitol (1 mg per body weight) was given instead of glucose showed no significant change in any of these parameters. *n* Indicates the number of observations and the number of animals.

Table 4 Effect of adrenaline and insulin on Na$^+$–K$^+$ contents in isolated rat soleus muscle

	Na$^+$ Content (μmol per g wet weight)		K$^+$ Content (μmol per g wet weight)	
Control	10.9±0.4 (7)		77.5±1.2 (7)	
		$P < 0.001$		$P < 0.001$
Adrenaline (10^{-5} M)	5.9±0.5 (6)		84.1±0.8 (6)	
		$P < 0.01$		$P < 0.001$
Adrenaline (10^{-5} M) + insulin (100 mU ml^{-1})	3.7±0.4 (6)		94.1±1.6 (6)	
		$P < 0.001$		$P < 0.005$
Insulin (100 mU ml^{-1})	8.5±0.5 (7)		85.1±1.7 (7)	

Groups of six or seven muscles were incubated for 90 min in 3 ml of Krebs–Ringer bicarbonate buffer containing 1 mM ^{14}C-sucrose with or without the additions indicated. They were then blotted, homogenised in 4 ml 5% trichloroacetic acid and the extract centrifuged. Aliquots of the clear supernatant were taken for flame photometry and measurement of ^{14}C activity. The Na$^+$–K$^+$ contents of the tissue compartments not available to ^{14}C-sucrose were calculated and expressed as μmol per g tissue wet weight. The results are given as mean ±s.e.m. with the number of observations in parentheses.

ibly related to the hypoglycaemia and the ensuing catecholamine release from the adrenals. *In vivo*, the combined action of adrenoreceptor agonists and insulin was additive. In two animals (250 g) the injection of 50 μg salbutamol produced a hyperpolarisation of 12.2 mV (from −66.2±0.6 [40 observations/2 muscles] to −78.4±0.5 [52/2]). The subsequent injection of 1 IU of insulin produced a further 4.6 mV hyperpolarisation (to −83.0±0.5 [64/2]).

As demonstrated earlier, stimulation of the active Na$^+$–K$^+$ transport, whether induced by insulin[6] or adrenaline[2], leads to a drop in the intracellular Na$^+$–K$^+$ concentration ratio (Table 4). Again, the actions of the two hormones on Na$^+$–K$^+$ distribution seems to be additive, indicating that even during a long-term exposure, their respective sets of cellular signals do not interfere with each other.

There is strong evidence that among the cellular signals initiated by adrenaline, cyclic AMP is important in eliciting a variety of effects produced by β_2-adrenoreceptor stimulation. Both cyclic AMP and dibutyryl cyclic AMP (in combination with the phosphodiesterase inhibitor, theophylline) stimulate the active electrogenic Na$^+$–K$^+$ transport[2]. Our observations are not readily compatible with the assumption that the action of insulin on Na$^+$–K$^+$ transport (judged by the measurement of active ion fluxes and cell Na$^+$–K$^+$ content) is secondary to a decrease in the concentration of cytoplasmic cyclic AMP. It seems reasonable to conclude that the system mediating active Na$^+$–K$^+$ transport is sensitive to two hormonal regulators sharing a final common path (see ref. 15).

The finding that a combination of β_2-adrenoreceptor agonist plus insulin can produce a hyperpolarisation that is significantly smaller than that of β_2-adrenoreceptor agonist alone does not correlate with the studies of ion transport and contents. This discrepancy may be explained if insulin not only induces a stimulation of a 3:2 coupled active Na$^+$–K$^+$ transport[6] but also increases the membrane permeability to Na$^+$ (ref. 8). A permeability increase could thus 'shunt' electrogenic ion translocation, or directly depolarise the membrane. However, we cannot explain why a change in the sequence of addition of insulin and adrenaline can produce significantly different results.

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Contractions of cat small intestinal smooth muscle in calcium-free solution

Allen W. Mangel, D. O. Nelson, John A. Connor & C. Ladd Prosser

Department of Physiology and Biophysics, University of Illinois, Urbana, Illinois 61801

A rise in internal Ca^{2+} is the signal which activates the contractile mechanism in muscle. In striated muscle there is a well developed sarcoplasmic reticulum which stores and releases the bulk of the Ca^{2+} involved in the contraction cycle[1,2], allowing the muscle to operate relatively independently of external calcium levels. In smooth muscle the sarcoplasmic reticulum system is much less extensive[3,4] and normal contractile activity is very sensitive to calcium levels in the bathing medium[5–7]. These findings, together with the small size of smooth muscle cells which minimises diffusion time from the plasma membrane to the contractile apparatus, have suggested to some investigators that the Ca^{2+} which enters the cell during Ca^{2+}-mediated action potentials is either sufficient by itself to activate contraction or is a necessary trigger for the further release of Ca^{2+} from internal stores[8–11]. This notion is given support by the observation that contractile activity rapidly dies out when smooth muscle preparations are exposed to calcium-free solutions. However, electrical activity is eventually lost in these solutions. We report here on spontaneous contractile and electrical activity, and evoked mechanical activity, recorded after prolonged exposure to calcium-free EGTA saline in intestinal smooth muscle of cat.

Segments of small intestine were removed from cats anaesthetised with α-chloralose and placed immediately into Krebs solution of the following composition (mM): NaCl, 118.5; KCl, 4.7; $MgCl_2$, 1.2; $NaHCO_3$, 23.8; KH_2PO_4, 1.2; $CaCl_2$, 2.5, and glucose 5.5 (ref. 12). External electrical recording techniques were used[11]. Mechanical recordings were made with an isometric tension transducer (Grass Instruments model FTO3C). Experiments were performed at 35–37 °C.

Normal electrical activity in cat small intestine consists of slow rhythmic depolarisations (slow waves) with spike potentials often superimposed near the slow wave peak. The slow depolarisation is maintained for 2 to 4 s and peak amplitude recorded intracellularly ranges between 16 and 40 mV in normal musculature[12]. Spike potentials seldom reach 0 mV in intracellular recordings. When gross contractions occur they are associated with spike activity.

Prosser *et al.*[13] showed that normal electrical activity in cat small intestine was eliminated by incubation in Krebs solution containing no added calcium and 5–10 mM EGTA ('calcium-free Krebs'). Following periods of electrical quiescence which ranged from 10 to 40 min, the muscle developed prolonged potentials in the calcium-free Krebs solution. Intracellularly recorded, the potentials were approximately 40 mV in amplitude. Thus the prolonged potentials apparently result from sodium ions traversing channels normally used by calcium.

Mechanical and electrical events in control Krebs, during the period immediately after exposure to calcium-free Krebs and after incubation for 40 min in calcium-free Krebs, are shown in Fig. 1*a*, *b*, *c*. The prolonged potentials developed in approximately 40% of the preparations tested, and rhythmic contractions, such as those shown in Fig. 1*c* and 1*d*, were displayed by 90% of these spontaneous preparations. The mean amplitude of the EGTA contractions was 65% of the control contractions, although some were larger than control activity. Calcium-free Krebs flowed continuously through the chamber during experiments.

To exclude the possibility that calcium might be leached from the mucosa into the interstitial fluid and act as an extracellular calcium store to the muscle cell, 30 preparations in which the mucosa was surgically removed were tested. The results of incubation of mucosa-free preparations in calcium-free saline were qualitatively the same as those obtained for intact preparations except the success rate of obtaining EGTA-induced activity was diminished in preparations devoid of the mucosa, possibly due to the more extensive manipulation of the tissue.

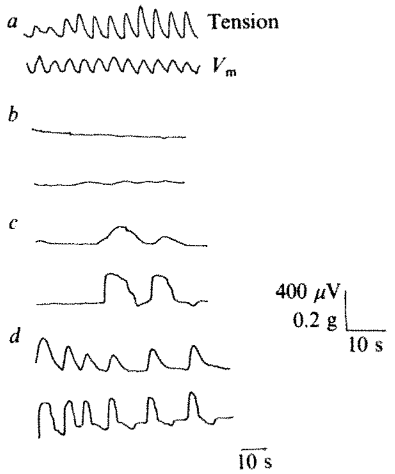

Fig. 1 *a*, Pressure electrode[11] recording of electrical activity (V_m, lower trace) and corresponding mechanical activity (upper trace) in control saline solution. *b*, Resulting activity following incubation for 7 min in calcium-free saline. *c*, Exposure for 40 min to calcium-free saline. *d*, Approximately 47 min exposure to calcium-free solution. Note change in time calibration.

Electrical stimulation could induce contractions in preparations remaining quiescent in calcium-free solution (Fig. 2). In some cases these preparations developed spontaneous electrical and contractile activity after stimulation. In other quiescent preparations barium chloride was applied and induced spikes with accompanying contractions (Fig. 3). Either treatment was effective even after preparations had been in calcium-free saline for over 2 h.

In the barium experiments the possibility exists of direct activation of the contractile apparatus by barium. However, this seems unlikely in view of the relative concentrations involved. Ebashi *et al.*[14] demonstrated that myosin B preparations from chicken gizzard and bovine aortic muscles are approximately 200 times less sensitive to barium than to calcium. Barium-induced contractions in calcium-free saline can be as large as control contractions, suggesting that internal barium levels would have to approximate 200 times internal calcium levels during peak contractions (~2 μM level for calcium-activated contractions). We would suggest, instead, that barium acts by supporting large action potentials[15] and that the plasma membrane depolarisation results in a release of internal calcium. The action of barium would then be similar to direct electrical stimulation on preparations in calcium-free saline.

Barrett and Barrett[16] have demonstrated the existence of a 'protected' extracellular compartment of calcium in frog skeletal muscle. The contents of this compartment were not removed by incubation of muscles in solutions with no added calcium plus 1 mM EGTA but were removed by high EGTA saline. To eliminate the possible presence of a 'protected' extracellular calcium compartment in cat small intestine, we incubated

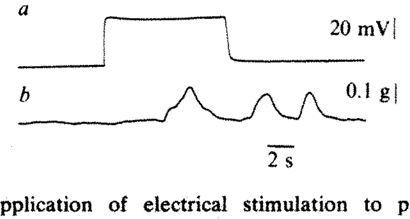

Fig. 2 Application of electrical stimulation to preparation remaining quiescent after exposure for 30 min to calcium-free saline. Preparation is mucosa free.

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mucosa-free preparations in 10 mM EGTA for 20 min and then in 30–50 mM EGTA for 30 min. Contractions could be elicited by electrical stimulation or barium chloride for the first 10 min in high EGTA. The final muscle contraction in high EGTA did not relax and tension thereafter showed a slow, steady increase, indicating that the muscle may have gone into contracture. This state was observed for periods of up to 20 min but may have lasted significantly longer. High EGTA concentrations might have induced contracture by large membrane depolarisations[16].

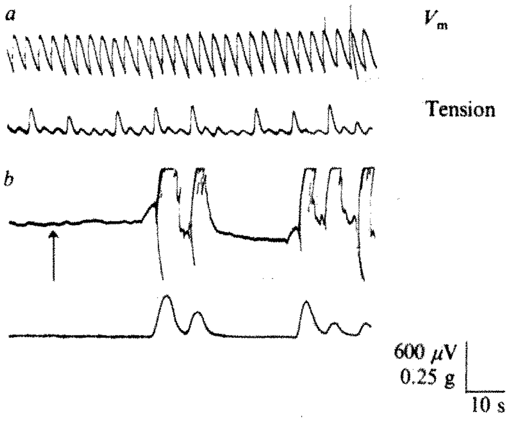

Fig. 3 *a*, Volume recording of electrical activity (upper trace) and corresponding mechanical activity (lower trace) in control saline solution from a mucosa-free preparation. *b*, Thirty seconds (at arrow) after the start of 3 mM $BaCl_2$ in calcium-free saline; the preparation was incubated in calcium-free saline for 175 min.

Our findings are similar to those of Somlyo *et al.*[4] who observed spontaneous contractions of turtle oviduct that persisted for ~30 min in calcium-free saline (4 mM EGTA). These investigators concluded that intracellular as well as extracellular sources of calcium can contribute to activation of contraction in smooth muscle. In contrast, others have reported that rhythmic mechanical activity is eliminated by EGTA[17,18]. It is possible that these observations were made during a time corresponding to the transition period between normal activity and EGTA-induced activity in our experiments.

In the calcium-free solutions it is unlikely that there is an appreciable inward-directed calcium gradient. The ability of prolonged potentials, barium spikes or electrical stimulation to induce contractile activity then suggests that an influx of calcium is not a prerequisite for contractions to occur and that internal stores of calcium which may be released by membrane depolarisation can support the contractile process. It is not clear to what extent this internal store of calcium participates in contractions in physiological conditions. Since the contractions we observed in calcium-free saline lasted for up to 1 h, it seems probable that a substantial portion of the activating calcium is recycled and therefore not readily accessible to extrusion mechanisms such as Na–Ca exchange.

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Control of cytoplasmic actin gel–sol transformation by gelsolin, a calcium-dependent regulatory protein

Helen L. Yin & Thomas P. Stossel

Hematology-Oncology Unit, Massachusetts General Hospital,
Department of Medicine, Harvard Medical School, Boston,
Massachusetts 02114

The peripheral cytoplasm of macrophages is involved in the control of locomotion, secretion and endocytosis, events common to many eukaryotic cells. During these activities, the cortical cytoplasm, which contains numerous actin filaments[1,2], appears to undergo reversible gel–sol transformations[3]: cycles of gelation and solation are demonstrable in suitably prepared macrophage extracts, and the gels contain tangled actin filaments[4]. These changes in consistency of cytoplasmic actin may regulate motile events in the macrophage periphery. Calcium in micromolar concentrations prevents gelation of crude macrophage cytoplasmic extracts[4], providing a possible link to abundant indirect evidence implicating calcium in the regulation of locomotion, secretion and endocytosis[5]. Similar calcium-sensitive gelation phenomena occur in crude cell extracts from diverse cell types and may have a relevance for control of cell movements in general[6–11]. Actin gelation results from the cross-linking of actin filaments (F-actin) by other proteins. In macrophages, a high molecular weight actin-binding protein (ABP) is the principal actin cross-linking protein[12]. Cross-linking of actin by these purified actin-binding proteins, however, is insensitive to changes in the calcium concentration[4,12], so that another factor must mediate the expression of a calcium effect. We have now isolated such a calcium-dependent regulatory protein from macrophages and call it gelsolin.

Rabbit lung macrophage cytoplasmic extracts, prepared in buffered sucrose solution containing protease inhibitors[12] and 5 mM EGTA, solidified to form gels when warmed in the presence of 0.1 M KCl. Addition of calcium chloride to micromolar-free calcium concentration inhibited gelation and caused solation of existing gels. Chelation of Ca^{2+} with EGTA restored gelation to the calcium-treated extract, ruling out calcium-dependent denaturation of the gelation components. The fluid expressed from gelled extract by centrifugation at 12,000*g* for 15 min at 25 °C, designated S2, did not gel by itself. However, it inhibited the gelation of rabbit muscle F-actin by purified macrophage ABP or chicken gizzard filamin in the presence of Ca^{2+}. Excess EGTA restored gelation. The experimental conditions used to demonstrate the Ca^{2+}-dependent regulator activity in S2 were as described in Table 1 for the purified gelsolin protein, and the results were qualitatively similar. The inhibitory activity in S2 was sensitive to trypsin digestion (1 mg ml^{-1} for 30 min at 25 °C) and boiling at 100 °C for 1 min.

The regulatory activity in S2 was purified by sequential ion-exchange chromatography and gel filtration (Fig. 1). The gelation inhibitory activity eluted from a DEAE-Sepharose column in one symmetrical peak at KCl concentrations between 0.10 and 0.15 M, and eluted in a single symmetrical peak from a Sephadex G-200 column. Based on the elution of globular proteins with known molecular weights from this column, the regulator protein had an apparent molecular weight (MW) of 160,000. The purification recovered 26–69% of the gelation inhibitory activity present in S2 and enriched the specific inhibitory activity 36- to 61-fold. Purification of the calcium-dependent inhibitory activity coincided with the enrichment of a

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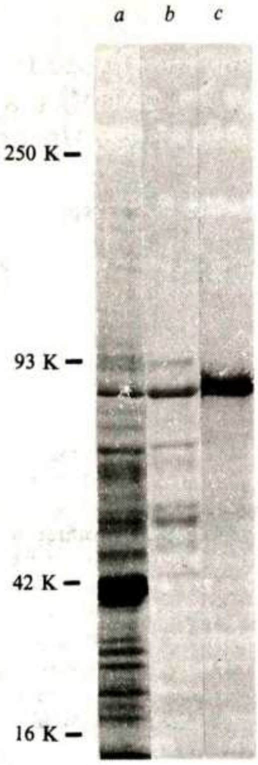

Fig. 1 Purification of gelsolin. All purification procedures were carried out in the cold. S2 (100 ml) was dialysed against 30 volumes of a solution containing 1 mM EGTA, 1 mM dithiothreitol, 0.5 mM ATP and 9 mM imidazole-HCl, *p*H 7.8. The dialysed sample was applied to a DEAE-Sepharose column (1.5 × 21 cm) equilibrated with the same buffer. The adherent proteins were eluted with a 500 ml linear 0–0.6 KCl concentration gradient. Fractions with gelation inhibitory activity (determined as described below) were pooled and solid ammonium sulphate was added to a final concentration of 306 mg ml^{-1}. The precipitated proteins were collected by centrifugation at 15,000*g* for 10 min, dissolved in 1 ml of a solution containing 0.8 M KCl, 1 mM EGTA, 5 mM dithiothreitol, and 10 mM imidazole-HCl, *p*H 7.5, and dialysed against the same solution. This solution was used for equilibration and elution of a Sephadex G-200 column (1.6 × 90 cm) to which the sample was applied. Calcium-dependent inhibition of actin gelation by the eluted fractions was measured by the sedimentation assay of Brotschi *et al.*[12]. Rabbit muscle F-actin (2 mg ml^{-1} prepared as in ref. 14, and filamin (0.05 mg ml^{-1}, prepared as in ref. 13) were incubated with the fractions to be tested in a solution of 0.1 M KCl, 20 mM imidazole-HCl, *p*H 7.2, 0.045 mM $CaCl_2$, for 30 min at 25 °C. Samples were centrifuged at 10,000*g* for 10 min at 20 °C and protein concentrations of the supernatant were determined by the method of Lowry[15]. The difference in protein concentrations between the initial reaction mixture and the final supernatant was a measure of the amount of protein incorporated into the sedimented gel pellet. The concentration of cross-linker in the control sample (without inhibitor) was adjusted to sediment about 50% of the total actin by 10 min, and the sedimentation rate was constant with time over this period. A decrease in actin plus cross-linker sedimented in the presence of added test fractions indicated an inhibition of gelation. The specific inhibitor activity was computed as: (mg protein sedimented in control − mg protein sedimented in sample with test protein)/mg test protein. Proteins from each purification step containing inhibitory activity were boiled for 1 min in 8 M urea, 10% SDS, 2% 2-mercaptoethanol and resolved by electrophoresis in 5–15% polyacrylamide gradient slab gels in the presence of SDS[16]. *a*, Macrophage S2 extract, 300 μg; *b*, DEAE-Sepharose fraction, 300 μg; *c*, Sephadex G-200 fraction, 50 μg. The subunit MWs (×10^{-3}) of standard proteins are indicated.

Table 1 Calcium-dependent reversible inhibition of actin gelation by gelsolin

	Treatment		
Proteins	First incubation	Second incubation	Gelation %
1. Actin, ABP	Ca^{2+}	—	100
2. Actin, ABP	EGTA	—	100
3. Actin, ABP, gelsolin	Ca^{2+}	—	0
4. Actin, ABP, gelsolin	EGTA	—	91
5. Actin, gelsolin	Ca^{2+}	—	0
6. Actin, gelsolin	EGTA	—	0
7. Actin, ABP, gelsolin	Ca^{2+}	EGTA	76
8. Actin, ABP, gelsolin	EGTA	Ca^{2+}	33
9. Actin, filamin	Ca^{2+}	—	100
10. Actin, filamin	EGTA	—	112
11. Actin, filamin, gelsolin	Ca^{2+}	—	0
12. Actin, filamin, gelsolin	EGTA	—	99
13. Actin, filamin, gelsolin	EGTA	Ca^{2+}	25

F-actin (2 mg ml^{-1}), actin cross-linking proteins (macrophage ABP, 0.024 mg ml^{-1} prepared as in ref. 12, or chicken gizzard filamin, 0.045 mg ml^{-1}) and gelsolin (0.025 mg ml^{-1}) were incubated in a solution containing 0.1 M KCl, 20 mM imidazole-HCl, *p*H 7.2, either 0.07 mM $CaCl_2$ or 1 mM EGTA, for 30 min at 25 °C. Samples no. 8 and 13 contained 0.2 mM EGTA. In a second incubation (40 min), EGTA was added to sample 7 to a final concentration of 1 mM, and $CaCl_2$ was added to samples 8 and 13 to 0.5 mM. An equivalent volume of buffer was added to the remaining tubes. Actin cross-linking was quantitated by the sedimentation assay of Brotschi *et al.*[12]. Data were pooled from three separate experiments, and gelation was expressed as per cent of that observed in paired control samples (without gelsolin). 100% gelation for the experiment shown in lines 1–6 was 0.7 mg ml^{-1} after a 10 min centrifugation, 1.1 mg ml^{-1} for line 8 and 0.9 mg ml^{-1} for lines 9–13.

91,000-MW polypeptide resolved by SDS polyacrylamide gel electrophoresis. This polypeptide was the major Coomassie blue-stained protein of the pooled G-200 activity peak (Fig. 1*c*). We therefore concluded that the 91,000 polypeptides are subunits of gelsolin and, based on the gel filtration data, suggest that the native molecule contains two of these subunits. Purified gelsolin, like the inhibitory factor in S2, was heat-labile, and conferred reversible calcium sensitivity to the gelation of actin filaments by actin cross-linking proteins (Table 1). Gelation of rabbit muscle actin by purified rabbit macrophage ABP was insensitive to Ca^{2+} (lines 1 and 2). Actin gelation by macrophage ABP became sensitive to Ca^{2+} when gelsolin was included: gelation was inhibited completely in the presence of calcium (line 3) but not in the presence of excess EGTA (line 4). Gelsolin by itself did not cross-link actin (lines 5 and 6). The inhibitory effect of gelsolin was partially reversible; gelation was largely restored to a calcium-treated sample by subsequent addition of sufficient quantities of EGTA (line 8). Similar calcium-dependent inhibition of gelation occurred when filamin was used as the actin cross-linking agent (lines 9–13). Inhibition of gelation

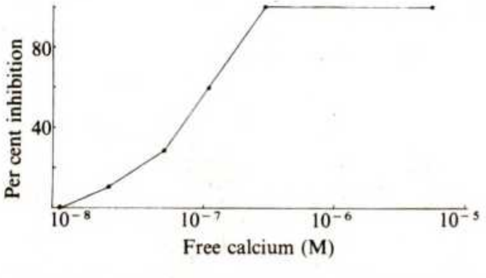

Fig. 2 Effect of calcium concentration on the inhibition of actin gelation by gelsolin. Gelsolin (0.03 mg ml^{-1}) was incubated with F-actin (2.0 mg ml^{-1}) and filamin (0.07 mg ml^{-1}) in a solution containing 0.1 M KCl, 20 mM imidazole-HCl, *p*H 7.2. The free Ca^{2+} concentration was varied by the use of Ca/EGTA buffers containing 0.4 mM EGTA, and calculated with the computer program of Perrin and Sayce[17], as described by Potter and Gergley[18].

Table 2 Effects of gelsolin on filamin–actin binding and actin sedimentability

Proteins	Conditions	Per cent of each protein sedimented Actin	 Filamin	 Gelsolin	Weight ratios of sedimented proteins Filamin : actin	 Gelsolin : actin
1. Actin, filamin	Ca^{2+}	92	100	—	1:18	—
2. Actin, filamin, gelsolin	Ca^{2+}	82	100	47	1:16	1:55
3. Actin	Ca^{2+}	85	—	—	—	—
4. Actin, gelsolin	Ca^{2+}	71	—	47	—	1:48
5. Actin, filamin	EGTA	95	100	—	1:18	—
6. Actin, filamin, gelsolin	EGTA	93	100	18	1:18	1:163
7. Actin	EGTA	91	—	—	—	—
8. Actin, gelsolin	EGTA	91	—	15	—	1:192

F-actin (1.9 mg ml^{-1}) was incubated for 30 min at 25 °C with gelsolin (0.06 mg ml^{-1}) and filamin (0.1 mg ml^{-1}) in a solution of 0.1 M KCl, 20 mM imidazole-HCl, *p*H 7.2. Either 0.07 mM $CaCl_2$ or 0.4 mM EGTA was present. The tubes were centrifuged at 93,000*g* for 1.5 h at 4 °C. Equivalent aliquots of the initial mixtures and pelleted proteins were analysed by polyacrylamide gel electrophoresis in the presence of SDS[16]. The intensities of the Coomassie blue-stained bands were determined by scanning with a densitometer (E–Z Apparatus Corp), and the amounts of each protein sedimented were expressed as a percentage of that present in the initial mixtures.

occurred instantaneously, and the extent of inhibition was proportional to gelsolin concentrations. Assuming a MW of 90,000 for gelsolin subunit, the molar ratio of gelsolin subunit to actin monomers (MW 42,000) was 1:166, and gelsolin subunit to ABP or filamin (MW 500,000) was 1:0.45 when gelation was maximally inhibited.

Figure 2 shows the concentration of Ca^{2+} required for inhibition of actin gelation by gelsolin. These concentrations of free Ca^{2+} also inhibit gelation of cytoplasmic extract of macrophages and are within the limits observed in living cells.

To determine the site of action of gelsolin, we examined its effects on the binding of cross-linker proteins to actin, and on actin sedimentability and viscosity in the absence of cross-linking proteins. The binding of filamin and F-actin was determined from the extent of co-sedimentation of the two proteins at high centrifugal force. Filamin alone did not sediment. The amount of filamin which co-sedimented with F-actin was not changed after incubation with Ca^{2+} at a concentration of gelsolin in excess of that required to produce maximal inhibition of gelation (Table 2, lines 1, 2). In fact, because the sedimentability of actin decreased in the presence of gelsolin (compare lines 1 and 2), the filamin to actin weight ratios in the pellet increased slightly in the gelsolin-containing sample, compared with the control. Therefore, inhibition of gelation did not result from a decrease in the number of cross-linker molecules bound to the actin filaments.

A small decrease in actin sedimentability was also observed when actin was incubated with gelsolin and calcium in the absence of filamin (compare Table 2, lines 3 and 4). No change occurred in the presence of EGTA (compare lines 5 and 6, 7 and 8). A direct calcium-dependent effect of gelsolin on actin was demonstrable by measuring the viscosities of F-actin solutions. When an F-actin solution (2.0 mg ml^{-1}) was incubated with gelsolin (0.03 mg ml^{-1}), a concentration which completely inhibited gelation of actin by filamin, the specific viscosity, measured with Cannon–Manning microviscometers (size 100), decreased by 30% in the presence of Ca^{2+}, but did not change in the presence of EGTA. The Ca^{2+}-dependent drop in specific viscosity was equivalent to a decrease in F-actin concentration from 2.0 mg ml^{-1} to 1.3 mg ml^{-1}.

Although the decreases in actin viscosity and sedimentability are consistent with shortening of actin filaments, it is clear from the small magnitude of the change that a substantial amount of gelsolin-treated actin remained in the polymerised state in conditions of complete inhibition of actin gelation.

Gelsolin may exert its effects by binding to actin in the presence of calcium. Gelsolin alone did not sediment under high-speed centrifugation. After incubation with F-actin and Ca^{2+}, about half of the added gelsolin co-sedimented with actin (Table 2, line 4). Addition of filamin did not change the proportion of gelsolin co-sedimented with actin (line 2), suggesting that gelsolin bound directly to actin and not filamin. In these experiments, a large amount of gelsolin, in excess of that required to dissolve actin gels, was used to render the polypeptide visible on stained polyacrylamide gels. Because of this excess, some of the added gelsolin remained in the supernatant. Furthermore, less actin sedimented in these conditions, and any gelsolin which might be bound to the non-sedimented actin would also remain in the supernatant. In the presence of EGTA, less than 20% of the gelsolin sedimented with actin, even though more actin was pelleted (Table 2, lines 6, 8). The ratios of pelleted gelsolin to actin were therefore much lower in the EGTA-treated samples than the calcium-treated samples.

These experiments have shown that gelsolin bound to actin in a Ca^{2+}-dependent manner, and decreased the viscosity and sedimentability of actin in the absence of cross-linking proteins. Inhibition of actin gelation by gelsolin may therefore be mediated by a direct effect of gelsolin on actin filaments. To explain complete inhibition of actin gelation following limited decrease in actin viscosity and sedimentability, we propose that gelsolin divides actin filaments between cross-linking points, thereby increasing the number of filaments. This mechanism of disrupting cross-linked networks is consistent with the established theory for polymer gels, which predicts that small changes in the cross-linker to polymer ratio near a critical point can abruptly effect sol–gel transformations[19]. Actin ABP and actin–filamin networks behave in accordance with this theory[12,20]. If gelsolin divides actin filaments, and the number of cross-links remains the same, the resulting increase in number of filaments effectively lowers the cross-link : polymer ratio, and causes dissolution of actin gels. The small number of gelsolin molecules required to inhibit gelation of actin is consistent with the idea that gelsolin can dissolve actin gels by causing a few breaks in actin filaments.

Our findings demonstrate that gelsolin, a heat-labile protein isolated from macrophages, dissolves actin networks in association with the limited breakdown of actin filaments. The expression of the regulatory function of gelsolin depends on variation in free calcium concentrations likely to occur in living cells. This is the first report of successful reconstitution of a Ca^{2+}-controlled actin gelation system from purified proteins, similar to that described in cytoplasmic extracts from diverse sources. We conclude that gelsolin is a physiological calcium-dependent regulator of macrophage cytoplasmic structure.

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Neurectoderm markers retained in phenotypical skeletal muscle cells arising from a glial cell line

Vanda A. Lennon & Susan Peterson

Neuroimmunology Laboratory, Departments of Neurology and Immunology, Mayo Clinic and Medical School, Rochester, Minnesota 55901

David Schubert

Neurobiology Laboratory, Salk Institute for Biological Studies, San Diego, California 92112

Differentiation *in vitro* of striated muscle from apparently non-muscle precursor cells has been reported in thymus reticulum[1], a fibroblast-like mouse embryo line[2] and in a neurone-like cell line derived from a rat brain tumour[3]. Also Tomozawa and Sueko reported the differentiation of a peripheral neurotumour clonal stem cell line into separate neuronal and glial cell types[4]. We report here the reproducible and stable phenotypic change of a well characterised rat glial cell line, B_9 (refs 5–7), into multinucleate contractile skeletal muscle. The B_9 line was derived from a nitrosoethylurea-induced brain tumour, contains S-100 and 14-3-2 proteins, is electrically non-excitable[5] and has a putative glial-specific surface antigen, G2 (ref. 6). It has been phenotypically stable in its laboratory of origin at Salk Institute since 1973. After being transported to the Mayo Clinic in Minnesota the cells began to change from cuboidal to elongate shape, fuse into contractile multinucleate fibres and express nicotinic acetylcholine receptors (AChR) on their plasma membranes. Striated myofibrils appeared in their cytoplasm. The first sign of mesectodermal differentiation in B_9 was the transitory appearance on its surface of Thy-1 which, in the rat, is a marker of thymocytes[8], certain brain cell lines[7], immature skeletal muscle[9,10], mammary myoepithelial lines[11] and fibroblasts[12]. This antigen was not detected in previous studies at Salk Institute[7]. The inductive influence is not yet known. We propose that transformation of a neurectodermal line into muscle may be evidence of the capacity of mammalian neurectoderm to give rise to skeletal tissues. The concept that skeletal muscle in different anatomical regions might have different embryonic origins could be relevant to the distribution of muscles affected by certain diseases in man.

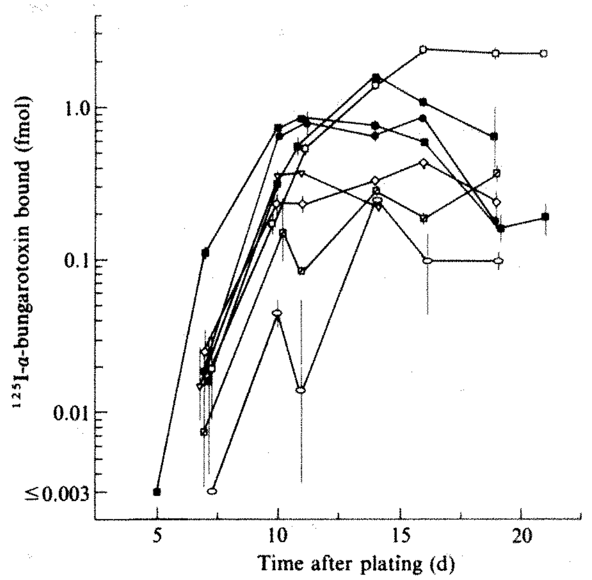

Fig. 1 Quantitation of nicotinic ACh receptors (AChR) on rat cell lines grown in microculture (2×10^3 cells in 0.2 ml DMEM/FCS, Microtest II plates, Falcon). Specific binding of α-bungarotoxin (α-BT, prepared and labelled with 5 mCi ^{125}I per 100 μg as described elsewhere[22]) was measured at multiple time points as follows. DMEM was withdrawn and 12 replicated wells washed once with HEPES-buffered DMEM (HBM) with 5% FCS. To 6 of the 12 wells was added 0.2 M carbamylcholine (CC). After 20 min at 21 °C an excess of ^{125}I-α-BT (4×10^{-9} M) was added to all 12 wells. Fluid was withdrawn after 90 min and the wells were washed three times with HBM/FCS, then one drop of 1 M KOH was added to each. After 15 min, the wells were swabbed with cotton-tipped applicators which were transferred to glass tubes for γ counting. Specific binding sites for ^{125}I-α-BT were calculated as: (mean c.p.m. without CC) − (mean c.p.m. with CC) × (mol standard ^{125}I-α-BT/c.p.m. standard ^{125}I-α-BT). Demonstration of antigenic determinants of nicotinic AChR in association with an α-BT binding macromolecule, extracted in non-ionic detergent from B_9M (subclone 33), distinguished this AChR from that of sympathetic ganglia type AChR[14]. Washed B_9M no. 33 cells (grown for 15 d in dishes of 150 cm^2 area) were extracted with 3% Triton X-100 (in 10 mM Tris, 100 mM NaCl, 1 mM EDTA, 5×10^{-4} M NaN_3 and 5×10^{-5} M PMSF). In the presence of an excess of ^{125}I-α-BT, a macromolecule binding α-BT was quantitated by chromatography on Sephadex G-100 (yield 2.34 fmol per cm^2 plate), as well as by precipitation from Triton X-100 extract[22] by sequential addition of rat antibodies against *Torpedo* electric organ AChR and goat anti-rat Ig (yield 2.18 fmol per cm^2 plate). Cell lines shown include: ●, L6 (mesenchymal muscle); ■, B_9M (B_9-derived; muscle phenotype) and six subclones of B_9M: ▽, 13 (predominantly cuboidal; thin myotubes); ○, 15 (mixed, elongate and cuboidal); ◇, 20 (mixed, cuboidal and elongate; large myotubes); ▨, 27 (predominantly cuboidal; less fusion of elongates); ☒, 31 (mixed elongate and cuboidal; many multinucleate myotubes); □, 33 (predominantly elongate, large myotubes). Glial cell lines B_{92} and B_9 are not shown, as they had no significant α-BT binding at any time (<0.01 fmol per well).

Glial lines B_9 and B_{92} (ref. 5) and a myogenic line, L6, from rat limb muscle[13], were transported by air at room temperature in plastic flasks containing Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS), penicillin G and streptomycin. B_9 and L6 were collected by Viokase treatment (20%, 37 °C, 15 min), and passaged every 7 days at low density (1.5×10^4 cells) in no. 3002 dishes (Falcon Labware) containing 5 ml DMEM with 10% FCS, penicillin G (20 U ml^{-1}) and streptomycin (0.02 mg ml^{-1}). B_{92} was collected by vigorous pipetting and similarly passaged. After the 7th passage, at 37 °C in humidified 12% CO_2/88% air, confluent cultures of B_9 were noted to have changed from cuboidal to fusiform shape. Many cells had fused to form multinucleate, vigorously contracting myotubes. At that time the laboratory had not received L6 or any other myogenic cell line. The changed cell line was renamed B_9M. Some cells were sent to California for continued passage and observation of phenotype; their progeny retained the skeletal muscle morphology on return to the original laboratory environment. Fresh stock of B_9 was sent from California for reference testing with B_9M and samples were cryopreserved after one subculture. After two passages in the new environment (with a different preparation of FCS) the second shipment of B_9 similarly became elongate and expressed pharmacological and antigenic markers of skeletal muscle (described below).

Cloning of B_9M at limiting dilution yielded 36 subclones (plating efficiency 47%). All were initially cuboidal; some became elongate and fused to form contractile myotubes; some remained predominantly cuboidal, but at high density all developed into a mixture of elongate and cuboidal. Specific binding sites for α-bungarotoxin (α-BT, indicative of AChR) were demonstrated on B_9M and on subclones of differing

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Table 1 Free amino acid analyses of B_9, B_9M and L6

Amino Acid	B_9	B_9M	L6
GABA	30; 23	27; 26	<0.1; <0.1
Ornithine	1.7; 2.1	2.4; 3.2	2.4; 3.2
Ethanolamine	4.0; 4.2	3.8; 4.2	<0.1; <0.1
Lysine	19; 21	19; 17	23; 22
Histidine	3.1; 4.3	3.7; 3.1	7.3; 8.9
Arginine	10; 9.0	9.0; 8.2	13; 15
Phosphoserine	3.1; 3.0	3.5; 3.8	7.6; 6.6
Phosphoethanolamine	60; 66	55; 60	46; 41
Taurine	116; 118	112; 117	92; 90
Aspartic acid	47; 49	48; 53	64; 61
Threonine	56; 61	66; 61	111; 105
Serine	20; 19	24; 29	34; 39
Asparagine	69; 64	65; 60	89; 86
Proline	36; 36	32; 33	64; 70
Glutamic acid	196; 186	201; 192	125; 123
Glycine	148; 155	145; 140	123; 135
Alanine	133; 131	130; 135	71; 65
Valine	8.3; 8.5	7.1; 8.6	27; 19
Isoleucine	6.6; 8.6	9.1; 8.0	18; 24
Leucine	9.6; 8.2	10; 11	31; 25
Tysorine	5.2; 6.5	8.0; 7.2	20; 23
Phenylalanine	18; 15	16; 17	32; 38
β-Alanine	1.9; 2.3	2.1; 3.0	<0.1; <0.1

Analyses are expressed as residues per 1,000. Free amino acids were extracted as trichloracetic acid-soluble residues from two separate preparations of each cell line in the exponential growth phase as described previously[16]. The content of GABA and β-alanine in B_9 and B_9M is characteristic of neurectodermally derived cells; lack of these two amino acids in L6 is characteristic of cells of mesodermal origin[16]. Although significant differences are apparent in the content of several other amino acids in the B_9 and L6 lines, these differences are not found consistently between other cells of neurectodermal and mesodermal origin.

morphology (Fig. 1). Subclones giving rise to abundant myotubes bound more α-BT. Myotubes of no. 33 were widest in diameter and in repeated experiments this subclone had approximately four times more AChR than the standard muscle line L6. Neither glial line B_{92} nor the original B_9 bound ^{125}I-α-BT. Demonstration of antigenic determinants of skeletal muscle-type AChR in association with an α-BT-binding macromolecule extracted from B_9M (Fig. 1 legend) distinguished this receptor from that of sympathetic ganglia-type AChR[14].

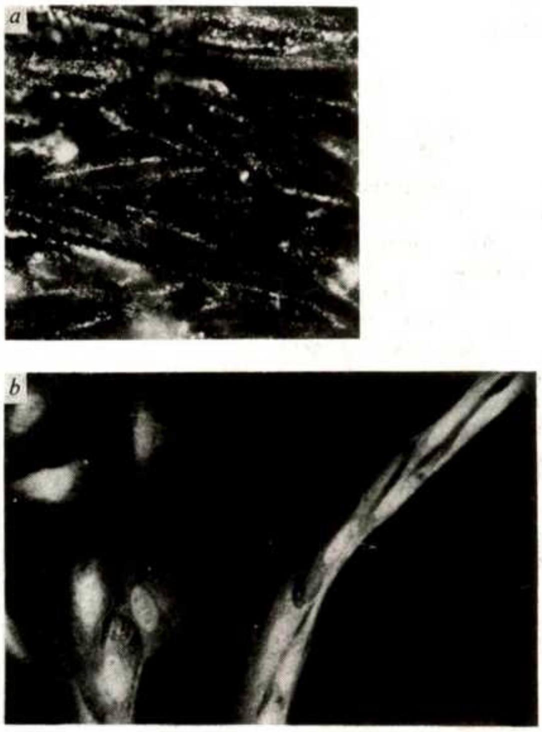

Fig. 2 For assay of Thy-1 by indirect immunofluorescence, cells were plated at 10^4 ml^{-1} into Petri dishes (1005, Falcon) containing round glass coverslips (no. 1 CGS, Propper Manufacturing Co.). ATS was used at a dilution of 1/50 followed by goat anti-rabbit IgG at 1/200 (Cappel Laboratories). *a*, In the living state the surfaces of elongate forms of subclone 33 of B_9M were Thy-1 positive. The characteristic coarse speckling of Thy-1 glycoprotein on developing myotubes in 10-day cultures is most prominent on cells of narrow diameter (×1,512). *b*, Acetone fixation revealed immunoreactive Thy-1 in the cytoplasm of cuboidal forms of the parental B_9M line and in aligned elongate pre-fusion cells 72 h after plating. Thy-1 glycoprotein is concentrated in the region of the nucleus in cuboidal forms and is more uniformly distributed in elongate forms (×1,512).

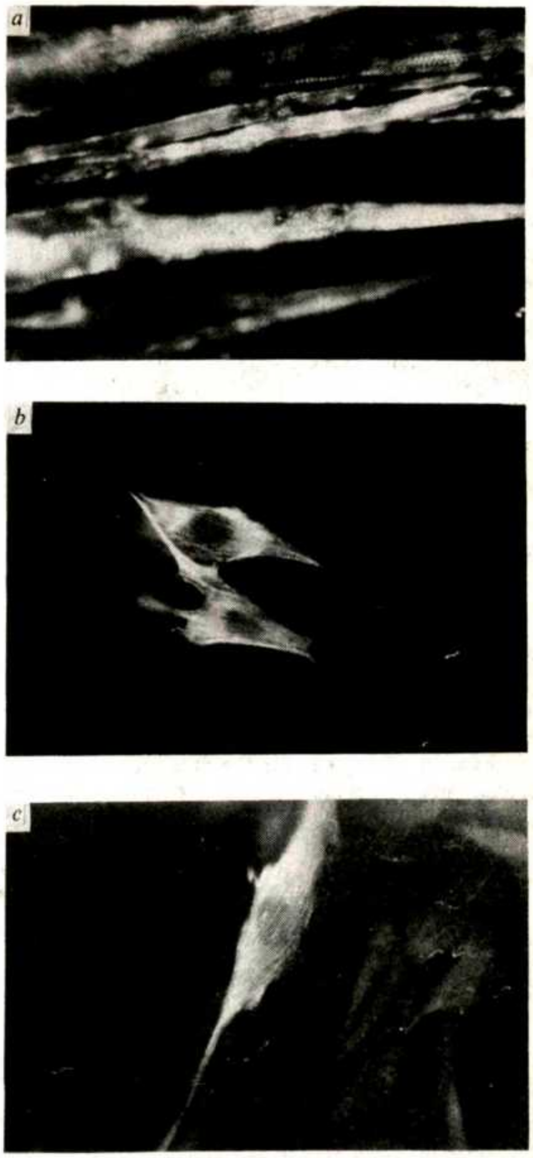

Fig. 3 Striated myofibrils characteristic of skeletal muscle were demonstrated in cytoplasm using serum (dilution 1/100) containing spontaneous autoantibodies reactive with contractile elements of skeletal muscle (obtained from a patient with myasthenia gravis). Indirect immunofluorescence used fluoresceinated goat anti-human IgG (Cappel Laboratories). *a*, Multinucleate L6 myotubes after 10 days in culture (×1,512); *b*, two aligned elongate B_9M cells after 72 h in culture (×1,512); *c*, positive elongate B_9M cell contrasts with negative background cells of more cuboidal morphology after 72 h in culture (×1,512).

Thy-1 glycoprotein was detected by indirect immunofluorescence using rabbit anti-rat thymocyte serum (ATS) described elsewhere[9]. No cell line exhibited fluorescence with ATS which had been absorbed by rat brain. In earlier studies of L6 and primary rat muscle cultures[9] we found Thy-1 on the surface of myoblasts and myotubes in early stages of fusion, but not on multinucleate myotubes. In the present study, L6 myoblasts, elongate forms of B_9M and its subclones and cuboidal cells contiguous with elongate forms were unequivocally positive (Fig. 2*a*). Acetone fixation revealed immunoreactive Thy-1 in the cytoplasm of cuboidal forms of B_9M (Fig. 2*b*). Small myotubes of B_9M and its subclones were most strongly positive for Thy-1; Thy-1 fluorescence on intermediate-sized myotubes was reduced (Fig. 2*a*) and multinucleate forms in 10-day cultures lacked detectable Thy-1. Recently transported B_9 lacked Thy-1, but after several passages, a few elongate Thy-1-positive cells appeared on top of the Thy-1-negative cuboidal monolayer.

Sera from certain patients with myasthenia gravis contain antibodies which react with contractile elements of striated muscle[15]. A myasthenia gravis patient's serum with this reactivity revealed cytoplasmic filaments in some mononucleate B_9M and L6 cells and striated myofibrils in multinucleate myotubes of both lines (Fig. 3). B_9 was negative for myofibril antigen.

Intracellular γ-aminobutyric acid (GABA) and β-alanine are found in cell lines derived from neurectoderm but not in cells derived from mesoderm[16]. To determine whether B_9M retained this neurectodermal marker, exponentially growing B_9, B_9M and L6 cells were analysed for free amino acids[16] (Table 1). B_9M and B_9 had very similar free amino acid compositions, but these differed appreciably from L6; B_9M and B_9 contained significant amounts of GABA and β-alanine whereas L6 characteristically lacked these two amino acids. Thus, the phenotypically changed B_9 metabolised amino acids in a manner similar to its parental cell line but distinct from mesoderm-derived skeletal muscle.

Experimental observations in lower vertebrate embryos indicate that certain mesenchymal elements of the head and neck arise from neural crest[17,18]. By grafting neural crest cells between quail and chick, LeLievre and LeDouarin[18] established that crest cells of the cephalic neural axis down to the 5th somite can give rise to mesenchymal derivatives (including skeletal muscle). If mammalian cephalic neurectoderm can also give rise to mesenchyme, this may explain the rare occurrence of striated muscle elements in primary tumours of the central nervous system (CNS) in man[19], and the derivation of clonal cell lines of skeletal muscle phenotype from tumours induced chemically in developing rat CNS (ref. 5, and D.S., unpublished observation). Rarely, tumours of skeletal muscle arise in the thymus[20], which is a pharyngeal pouch derivative. Thymic mesenchyme is predominantly neural-crest derived[21] and it is likely that its skeletal muscle elements[22,23] are also of neural crest origin. The thymus is prominently involved in the immunopathology of myasthenia gravis; it is probably the major site of autoimmunisation against AChR which is the cause of failure of neuromuscular transmission in this disease[23]. If AChR of thymic myogenic cells and cranial muscle were to share antigenic determinants unique to AChR of neurectodermal origin, the head and neck muscles, which are commonly involved in myasthenia gravis, might be the preferential targets of circulating antibodies with specificities restricted to neurectodermal determinants of AChR. Our observation of skeletal muscle differentiation from a neurectodermal cell line of rat suggests that the cephalic neural crest of mammals, like that of lower vertebrates[17,18], might have myogenic potentiality. It remains to be determined whether mammalian neurectoderm contributes to skeletal structures of the head and neck in normal histogenesis.

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Note added in proof: Striated muscle fibres are known to exist in normal pineal glands, including human[24]. Recent documentation of the electrophysiological and pharmacological properties of skeletal muscle fibres arising from primary cultures of rat pineals[25] further supports our hypothesis of the myogenic potentiality of mammalian neurectoderm.

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The production of the arachidonate metabolite HETE in vascular tissue

James E. Greenwald, Joseph R. Bianchine & Lan K. Wong

Department of Pharmacology, The Ohio State University, College of Medicine, Columbus, Ohio 43210

Blood platelets contain two enzyme systems which oxygenate arachidonic acid (AA). First, a cyclooxygenase[1] which metabolises AA into the biologically active prostaglandin endoperoxides (PGG_2 and PGH_2) which are precursors for the platelet prostaglandins PGE_2, $PGF_{2\alpha}$ and thromboxane A_2 (TxA_2). And second, a lipoxygenase that metabolises AA into 12-*L*-hydroperoxy-5, 8, 10, 14-eicosatetraenoic acid (HPETE)[1,2]. HPETE is a labile intermediate which is nonenzymatically reduced in aqueous environments to a more stable metabolite, 12-*L*-hydroxy-5, 8, 10, 14-eicosatetraenoic acid (HETE). HPETE is produced in significant quantities by aggregated platelets, but its exact biological role is not known. Turner *et al.*[3] has suggested that HETE may be a mediator of neutrophil chemotaxis. Vane and coworkers demonstrated that 15-hydroperoxy-5, 8, 10, 14-eicosatetraenoic acid[4,5] as well as HPETE were potent inhibitors of prostacyclin (PGI_2), the major arachidonate metabolite in vascular tissue. These data suggest that HPETE, if present in vascular tissue, might act as endogenous regulator of PGI_2 production. Using radiochemical techniques and mass spectrometry we have now identified a lipoxygenase enzyme in vascular tissue (rabbit aorta) in which biosynthesis of HETE also occurred. The detection of HETE in this suggests a potential role of the lipoxygenated product as an endogenous regulator of PGI_2 biosynthesis.

Rabbit aorta was cut into small rings (3–5 mg wet weight) and incubated with ^{14}C-labelled AA at 37 °C for 5 min. After incubation, the reaction medium was acidified with 1.0 M formic acid and extracted with ethyl acetate. The radioactive products were partitioned into the organic phase which was then evaporated to dryness under nitrogen. The products were resuspended in ethyl acetate/methanol (1 : 1) and subjected to TLC.

As shown in Fig. 1*a* and *c*, initial incubations of aortic rings yielded an unknown radioactive peak with an R_F value very similar to ricinoleic acid when developed in solvent system I or solvent system II. In a second experiment, indomethacin at a concentration (10^{-4} M) which completely inhibits the cyclooxygenase enzyme was added to the incubation medium, and production of this unknown arachidonate metabolite was stimulated by 50% (Fig. 1*b*, Table 1). From these data it can be concluded that this compound is not a cyclooxygenase product.

AA is prone to nonenzymatic oxidation and this mechanism might account for the unknown component. To differentiate between nonenzymatic and enzymatic oxidation of AA, aortic rings were preincubated with eicosatetraenoic acid (ETYA),

Table 1 Effects of inhibitors on HETE production in rabbit aorta

Inhibitors	HETE (pmol)	% Change from control
—	125.47*	+55
Indomethacin (1×10^{-4} M)	194.31*	
—	107.86†	−61
ETYA (1×10^{-4} M)	42.08†	
—	149.18‡	−48
PCMB (2×10^{-3} M)	78.03‡	

* An average of three animals.
† An average of two animals.
‡ An average of five animals.

which inhibits both lipoxygenase and cyclooxygenase[1]. As shown in Table 1, ETYA (10^{-4} M) completely blocked the formation of this compound, suggesting an enzymatic (lipoxygenase) pathway for the formation of this unknown compound.

If the unknown were HETE, selective inhibition of lipoxygenase would be expected to abolish the formation of this compound and desuppress the biosynthesis of PGI_2. Nugteren[6] found that para-chloromercuribenzoate (PCMB) was a selective inhibitor of human platelet lipoxygenase at 2×10^{-3} M. In our studies, incubation of PCMB (2×10^{-3} M) with aortic rings (Fig. 1*d*, Table 1) caused 50% inhibition of formation of the unknown compound, but no stimulation of PGI_2. PCMB tested at various concentrations with lipoxygenase from washed human platelets did not selectively inhibit HETE production—PCMB at 2×10^{-3} M inhibited formation of both HETE and PG (data not shown).

We further characterised the unknown component by gas chromatography–mass spectrometry (GC–MS) analysis. The TLC zone of interest was scraped and eluted with methanol. This extract was concentrated and derivatised with diazomethane followed by trimethylsilylation[1]. The sample was then analysed by selected ion monitoring on a Hewlett Packard model 5985 GC-MS system. Characteristic ions of HETE at *m/e* 391 (M-15), *m/e* 295 (base peak) and *m/e* 225 were observed at the expected retention time. These data show the unknown component to be HETE.

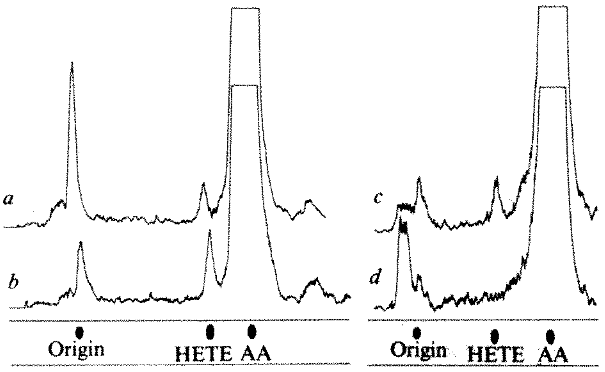

Fig. 1 TLC radiochromatograms of arachidonic acid metabolites in rabbit aorta. Rabbits (2–4 kg) were each given intravenously 1,000 units of sodium heparin to avoid blood clotting and anaesthetised with sodium pentobarbital. The animals were killed and their aortas excised and cut into small rings (4–5 mg per ring) in 50 mM Tris-HCl (*p*H 7.5) at 4 °C. Each incubation was carried out on 80–90 mg tissue with 7 nmol ^{14}C-labelled AA and 0.5 ml *p*H 7.5 Tris buffer in the presence or absence of a drug. The incubation was allowed to proceed for 5 min with shaking at 37 °C in a waterbath. Then the reaction was quenched with 1.0 M formic acid, extracted twice with three times its own volume of ethyl acetate and subjected to TLC. Authentic standards of PG and fatty acid were chromatographed in parallel with unknowns. Zones of radioactivity were detected by a Packard radioscanner and by iodine vapour visualisation. The zones were collected for liquid scintillation counting. *a*, An incubation in the absence of any drug using TLC system I (chloroform/methanol/acetic acid, 60:40:3). *b*, An incubation in the presence of 1×10^{-4} M indomethacin using TLC system I. *c*, An incubation similar to *a* but using TLC system II (ethyl acetate/2,2,4-trimethylpentane/water, 75:75:100). *d*, An incubation in the presence of 2×10^{-3} M PCMB using TLC system II.

Since the intermediate HPETE is an inhibitor of PGI_2 biosynthesis, the lipoxygenase enzyme in rabbit aorta may have a role in modulating PGI_2 production in vasculature. A high activity of lipoxygenase would suppress PGI_2 formation and result in vasoconstriction, and a low level of lipoxygenase would favour PGI_2 biosynthesis and cause vasodilation. Our findings suggest a physiological role of the lipoxygenase in the cardiovascular system.

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Tumour promoter induces anchorage independence irreversibly

Nancy H. Colburn, Brigitte F. Former, Katherine A. Nelson & Stuart H. Yuspa

In Vitro Pathogenesis Section, Laboratory of Experimental Pathology, National Cancer Institute, Bethesda, Maryland 20014

Tumour-promoting phorbol esters elicit a variety of molecular responses from cells in culture[1–3]. Phorbol esters are also active as promoters of neoplastic transformation in 10T1/2 mouse fibroblasts, previously initiated by polycyclic aromatic hydrocarbons[4] or UV[5] or X-ray irradiation[6]. As many *in vivo* studies of the tumour-promoting activity of phorbol esters have been carried out in mouse skin[7], it seems desirable to use mouse epidermal cell lines to study the mechanism of tumour promotion *in vitro*. Mouse epidermal cell lines would be particularly useful if they responded to phorbol esters by progressing towards a neoplastic phenotype. We have previously reported the development of cell lines derived from primary mouse epidermal cultures after carcinogen or solvent exposure[8]. Some of these cell lines remained non-tumorigenic for many passages and failed to form colonies in soft agar (frequency less than 10^{-4})[9]. We next asked whether some of these non-tumorigenic cells might respond to tumour promoters like 'initiated' or 'post-initiated' cells *in vivo* by progressing towards a neoplastic state[9,10]. We report here the identification of three epidermal cell lines which respond to tumour-promoting but not to non-promoting phorbol esters by irreversibly acquiring capacity to grow in soft agar. As anchorage-independent growth characterises malignant cells derived from a variety of sources including mouse epidermis[8,11], this response to phorbol esters may be analogous to a late stage of tumour promotion *in vivo*.

Primary epidermal cell cultures[12] were prepared from newborn BALB/c mice[8] or non-inbred Sencar mice[9] which had been bred for sensitivity to a 7,12-dimethylbenz (*a*) anthracene (DMBA)/12-*O*-tetradecanoyl phorbol-13-acetate (TPA) initiation-promotion scheme of skin carcinogenesis[13]. Cell lines were derived after exposure to carcinogen or to solvent alone as previously described[8,9]. All of these cell lines retained epidermal-specific cell-surface antigen (N.H.C. *et al.*, in preparation).

Table 1 Induction of growth in 0.33% agar medium after exposure to TPA

		No. of colonies per 10^4 cells			
		10% Serum		20% Serum	
Cell line	Initial treatment	Solvent control	TPA	Solvent control	TPA
JB-6	Solvent	<1	696	84	3888
D-2	DMBA	<1	24	84	756
JB-10	MNNG	<1	72	36	264

JB-6 and JB-10 cell lines were derived from solvent or MNNG-treated BALB/c primary cultures as described previously[8,9]. The D-2 line was derived from DMBA-treated (0.01 μg ml^{-1}, twice a week for 3 months) Sencar primary cultures. Cells were grown in monolayer culture in Eagle's minimal essential medium containing 10% fetal calf serum (FCS) and antibiotics and replated once a week[8]. Cells were suspended in 0.33% Difco agar medium[8] containing solvent alone (0.1% DMSO) or TPA at 0.1 μg ml^{-1} (1.6×10^{-7} M) and layered at 10^4 cells per 60-mm dish over a 0.5% agar base. The temperature of agar medium when mixed with phorbol esters was less than 40 °C. Assays were carried out in duplicate at both 10% and 20% FCS concentrations without refeeding and colonies were counted at 14 days. Cells were assayed at the following passages: JB-6: 35–51; D-2: 10–24; JB-10: 31–39. Each value is the mean for 3 to 8 separate experiments in which the s.e.m. ranged from 5 to 15% of the value.

While some lines were tumorigenic and formed colonies in 0.33% agar[8], others failed to grow in agar (in 10% serum) for at least 39 passages[8,9]. Twenty-one lines of the latter group were screened for colony formation in agar in response to tumour promoters. Three responders were identified including BALB/c lines JB-6, initially exposed only to solvent[8], and JB-10, initially exposed to *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG)[8]; and a Sencar line D-2, initially exposed to DMBA (N.C., unpublished). As with all anchorage-dependent mouse epidermal lines tested so far, JB-6 and JB-10 did not give rise to tumours after injection into syngeneic newborns at passages 57 and 15 respectively (refs 8, 9 and unpublished).

When exposed to the tumour-promoting phorbol ester TPA in agar medium, these three non-tumorigenic cell lines showed anchorage-independent growth (Table 1). Colony induction occurred at serum concentrations of 10% and 20%, with greater sensitivity for detection of induced colonies and higher background at 20% serum. This background may reflect weak colony inducing activity of serum growth factor(s) in agar medium. One growth factor, epidermal growth factor (EGF), has been reported to enhance skin tumour yield in 3-methylcholanthrene-treated mice[14]. Recent results (unpublished) have shown that EGF and sarcoma growth factor of Todaro and coworkers[15] will induce growth in agar by JB-6 cells. Previous findings indicated that epidermal cell lines which formed colonies at frequencies less than 1 per 10^4 in 10% serum in soft agar consistently turned out to be non-tumorigenic[8,9]. In contrast, tumorigenic lines usually formed colonies at frequencies greater than 200 per 10^4 in 10% serum. Hence, it seems likely that this TPA-dependent increase in anchorage independence represents induction of tumour cell phenotype. Of the three cell lines, JB-6 showed the highest response to TPA with 39% of the cells giving rise to colonies in 20% serum. To acquire this responsiveness, the JB-6 line required some 30 or more passages in culture. Cells from earlier passage (for example 18, 20, 28) showed no detectable response to TPA in agar (data not shown). Thus whatever 'initiation' or post-initiation events occurred in this solvent-treated line, they did so independently of exposure to added carcinogen and required considerable time in culture. In contrast the TPA responsiveness of D-2 and JB-10 appeared in earlier passages (10 and 15 respectively) and may have resulted from carcinogen exposure during primary culture (see Table 1).

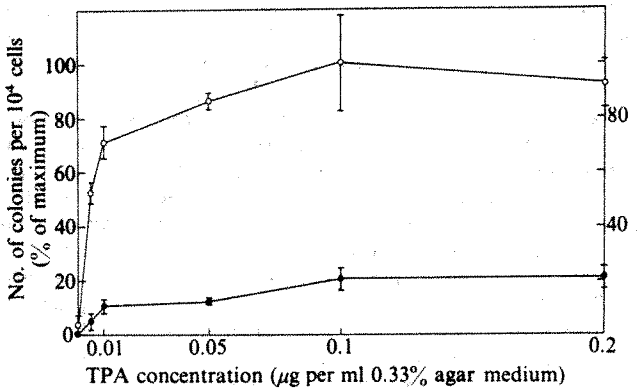

Fig. 1 Effect of TPA concentration on colony formation in soft agar. Assay of growth in 0.33% agar medium was carried out as described in the Table 1 legend. TPA concentration ranged from 0.005 to 0.2 μg ml^{-1} (8×10^{-9} M–3.3×10^{-7} M). JB-6 cells were assayed at passages 35 to 51. Each point represents the mean of 4 to 16 experiments. Vertical bars indicate s.e.m. Colony forming efficiency is expressed as a per cent of the maximum induced value (that obtained with 0.1 μg ml^{-1} TPA in 20% serum). This form of expression allows the combining of data from a number of experiments as colony forming efficiency varies with serum batch (unpublished). ●, 10% serum; ○, 20% serum.

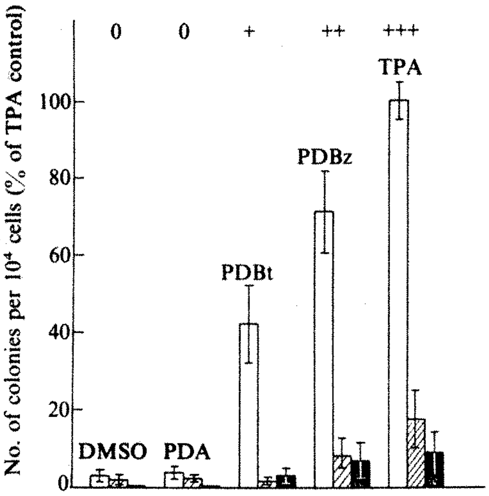

Fig. 2 Correlation of colony inducing activity with tumour-promoting activity of phorbol esters. Assay of growth in 0.33% agar medium was carried out as described in the Table 1 legend. All phorbol esters were used at a concentration of 0.1 μg per ml agar medium and a dimethyl sulphoxide (DMSO) concentration of 0.1%. The rank ordering of tumour-promoting activity of phorbol esters indicated by 0 to +++ is based on Baird and Boutwell[16]. Open bars: JB-6, 20% serum. Shaded bars: D-2, 20% serum. Solid bars: JB6, 10% serum. PDBz, phorbol-12,13-dibenzoate; PDBt, phorbol-12,13-dibutyrate; PDA, phorbol-12,13-diacetate; DMSO, dimethyl sulphoxide control.

Figure 1 shows the dependence of colony induction on TPA concentration. TPA at concentrations from 0.005 to 0.2 μg ml^{-1} induced colony formation with maximum induction occurring at 0.1 μg ml^{-1} (1.6×10^{-7} M). The dose–response curves are of similar shape in 10% or 20% serum but absolute colony forming efficiency is 4–5-fold higher in 20% serum. TPA at a concentration as low as 10^{-10} M has been found to induce colony formation in JB6 cells grown in agar medium containing 20% serum (data not shown).

Figure 2 indicates that other tumour-promoting phorbol esters but not non-promoting phorbol esters induced anchorage-independent growth in JB-6 and D-2 cells. For JB-6 at 20% or 10% serum the order of colony inducing activity was as follows: TPA > phorbol dibenzoate > phorbol dibutyrate, with the non-promoter phorbol diacetate showing no inducing activity (values identical to solvent control). In D-2 cells only TPA and phorbol dibenzoate induced colony formation. The colony inducing activity paralleled the tumour promoting activity reported for these phorbol esters by Baird and Boutwell[16].

Many changes induced by tumour-promoting phorbol esters in normal cells are transient and tend to mimic the neoplastic phenotype[17]. These changes include loss of cell-surface fibronectin[3] and induction of high levels of plasminogen activator[18] and ornithine decarboxylase[19–21]. Many of the phenotypic changes become permanent during pre-neoplastic progression at the time of conversion to overt malignancies often under the influence of promoters[22]. Thus if the TPA responsive cell lines represent a late stage in tumour promotion, one would expect promoter-induced anchorage independence to be irreversible.

After induction by TPA in agar, colonies were removed with a capillary tube and grown in monolayer culture to yield cell lines, each derived from a single agar colony. The cell lines were then suspended in 0.33% agar medium in the absence of TPA to determine whether the cells retained the capacity for anchorage-independent growth. As shown in Table 2, all of the 11 lines obtained formed colonies in soft agar at frequencies ranging from 96 to 1,992 per 10^4 cells in 10% serum and from 678 to 4,968 per 10^4 cells in 20% serum. Even the lower values were many times higher than those found for the untreated control. The original values obtained for colonies induced in the presence of TPA (TPA-treated control) are presented for comparison. The values for agar derived colonies ranged from 15% to 300% of the original TPA induced values and remained undiminished for at least 2 months following the colony isolation. Cell lines derived from the D-2 line (see Table 1) after induction of anchorage independence by TPA were also found to be stable for growth in soft agar (data not shown). The retained values for the D-2 derived lines were 7–11-fold higher than the original TPA-induced values. Retention of anchorage independence by TPA treated JB10 cells has not yet been tested.

The mechanism of the agar colony response to tumour promoters has yet to be elucidated. Studies in our laboratory (ref. 22, and manuscript in preparation) suggest that TPA induces a shift in anchorage independence rather than selecting for pre-existing anchorage-independent cells, as colonies form from single JB6 cells (plated one per well in soft agar) in response to TPA. Because induction of anchorage independence by tumour-promoting phorbol esters seems to occur rapidly and irreversibly in the JB-6 and D-2 epidermal cell lines, the process may be analogous to a late phase of tumour promotion *in vivo* in which TPA apparently converts benign premalignant lesions to malignant tumours irreversibly[22,23].

Table 2 Retention of anchorage independence in the absence of TPA

Cell line	Derivation Cells	Passage	No. of colonies per 10^4 cells 10% Serum	20% Serum
JB6		62–74	7	128
JB6 + TPA (0.01 μg ml^{-1})		62–74	649	2,073
T61	JB6	70	138	1,410
T62	JB6	70	1,992	3,504
T63	JB6	70	96	1,050
T64	JB6	70	824	2,784
T2111	JB6 C121	23	216	678
T2121	JB6 C121	23	852	3,594
T2122	JB6 C121	23	432	1,848
T2123	JB6 C121	23	162	1,632
T2124	JB6 C121	23	756	4,968
T2125	JB6 C121	23	1,050	2,796
T2126	JB6 C121	23	492	2,820

Cells were suspended in 0.33% agar medium as described in the legend to Table 1. Except for the TPA-treated controls (JB6 + TPA), all cell lines were grown in agar in the absence of TPA. Lines T61–T64 were derived from single colonies induced in soft agar after exposure of JB-6 cells to TPA (0.01 μg ml^{-1}) in 10% serum. Lines T2111–2126 were obtained from colonies produced after identical exposure of a clone derived (by growth from single cells in standard medium) from the JB-6 line, passage 50 and designated JB6 Clone 21. Cell lines derived from soft agar colonies were grown for 2 passages in monolayer culture (2–3 weeks) before assaying for retention of anchorage independence. Clone 21 yielded values for untreated controls and TPA-treated controls which were about 20% higher than the respective values for JB-6. Cell lines derived from background colonies (without TPA) in 20% serum retained colony forming frequencies similar to the original background levels shown. Each value is the mean for 2 experiments run in duplicate. The differences from earlier passages (Table 1) for solvent and TPA treated JB-6 controls may be attributable to a change in serum batch.

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Tumour promoters enhance anchorage-independent growth of adenovirus-transformed cells without altering the integration pattern of viral sequences

Paul B. Fisher, Karoline Dorsch-Häsler,
I. Bernard Weinstein & Harold S. Ginsberg

Cancer Center/Institute of Cancer Research,
Division of Environmental Sciences and Department of Microbiology,
Columbia University/College of Physicians and Surgeons,
New York, New York 10032

Carcinogenesis often involves interaction between multiple factors and proceeds through several discrete steps[1–4]. In mouse skin two distinct phases, 'initiation' and 'promotion', have been described[2]. The most potent tumour-promoting agents in mouse skin are 12-*O*-tetradecanoylphorbol-13-acetate (TPA) and related diterpenes[3–5]. A significant experimental advance in studies on the mechanism of action of tumour promoters has been the demonstration that they induce specific biochemical and biological effects in cell culture[6–8]. These effects include phenotypic changes that mimic those seen during transformation; modulation of differentiation; enhancement of the expression of markers of transformation in cells that are already transformed; and an increased yield of transformed foci in cultures exposed to chemical carcinogens or radiation[6–11]. We have previously reported that TPA also increases the yield of transformed foci in rat embryo cultures previously infected with a mutant of type 5 adenovirus[12]. When foci of adenovirus-transformed cells are picked, cloned and serially passaged, they

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undergo progressive changes, the most notable of which is acquisition of anchorage-independent growth[13]. The present study demonstrates that although TPA enhances the acquisition of anchorage-independent growth in adenovirus-transformed cells (and not in normal cells), this alteration in growth properties is not due to a change in the integration pattern of adenovirus DNA sequences in the genome of the host cell.

Normal secondary rat embryo (2°RE) cultures were infected with a mutant of type 5 adenovirus, H5ts125. This mutant was used because at 37 °C it is defective in DNA replication and has a higher transforming efficiency than wild-type virus[14]. In some experiments, the cultures were exposed to chemical carcinogens just before virus infection[12]. Epithelioid foci characteristic of adenovirus transformation were picked 5 to 6 weeks later, cloned and then passed in serial culture[12,13]. The presence of adenovirus DNA sequences in all of the isolated epithelioid clones was demonstrated by reassociation kinetics of ^{32}P-labelled Ad5 DNA *Hin*dIII restriction fragments and excess unlabelled transformed cell DNA[15]. We found that during early passages (<10) these clones had a low or negligible capacity to grow in agar, whereas at later passages (>30); they spontaneously acquired the ability to grow in agar, although with varying cloning efficiencies[13].

Although TPA does not induce growth in agar of normal 2°RE cells, it causes an appreciable enhancement of the agar cloning efficiency of the adenovirus-transformed clones (Table 1). With certain early passage adenovirus-transformed clones, growth in agar was undetectable unless TPA was added. With later passages, these clones spontaneously acquired the ability to grow in agar but their growth in agar was significantly enhanced by TPA. Similar results were obtained with 14 other clones of adenovirus-transformed cells[13]. The E11-E (assayed before 10 serial passages) and E11-L (assayed after 30 serial passages) clones have been studied in greater detail. Concentration curves demonstrated that 3 ng ml^{-1} was the optimal concentration of TPA (in agar) to effect anchorage-independent growth; a half-maximal effect was obtained with 1 ng ml^{-1} (1.6×10^{-10} M). Specificity of the TPA effect was demonstrated by the fact that 200 ng ml^{-1} of either phorbol or 4α phorbol-12-13-didecanoate (4αPDD), which are inactive as tumour promoters on mouse skin[3-5], failed to enhance growth in agar.

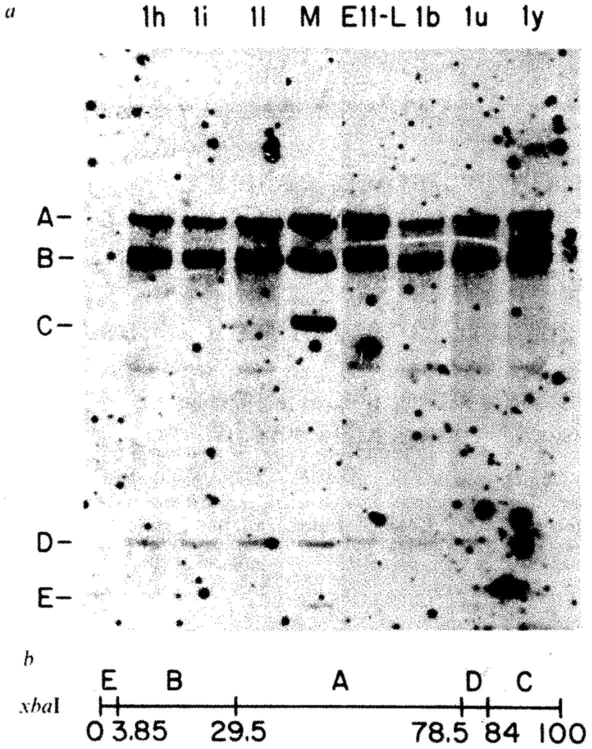

Fig. 1 *a*, Integration pattern of Ad5 DNA in a cloned population of H5ts125-transformed 2°RE cells (E11-L) and in subclones of E11-L isolated from agar either containing or lacking 100 ng ml^{-1} TPA. Confluent monolayers were grown from each subclone in low Ca^{2+} medium. DNA was isolated as described by Pellicer *et al.*[24] and 5 μg of DNA from each clone was cleaved with the restriction endonuclease *Xba*I. The DNA was electrophoresed in 0.7% agarose gels and then transferred to cellulose nitrate sheets according to a procedure slightly modified from the method of Southern[22]. Ad5 DNA labelled by nick-translation with ^{32}P to a specific activity of $2-4 \times 10^8$ c.p.m. per μg DNA[24] was used as a probe for filter hybridisation. The slot labelled M contains an artificial mixture of 5 μg DNA isolated from 2°RE cells and 5×10^{-5} μg purified Ad5 DNA cleaved with *Xba*I restriction endonuclease. The bands on the autoradiographs are identified as A–E. *b*, *Xba*I restriction map of Ad5 DNA.

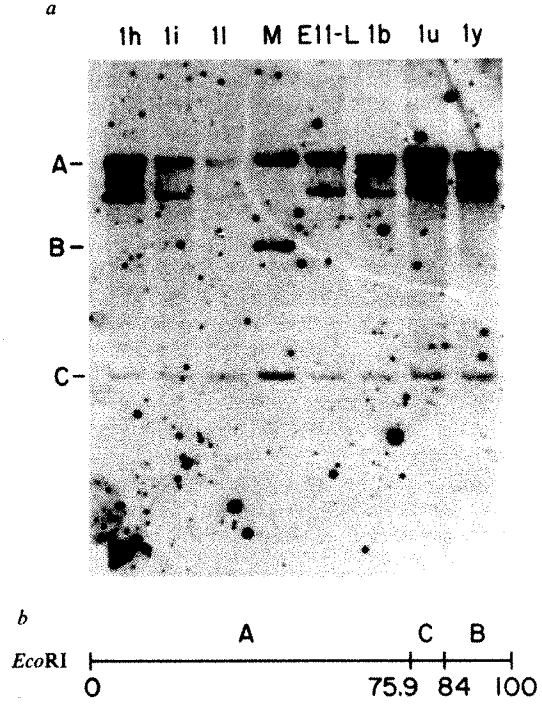

Fig. 2 *a*, Integration pattern of Ad5 DNA in a cloned population of H5ts125-transformed 2°RE cells (E11-L) and in subclones of E11-L. 5 μg of DNA was cleaved with *Eco*RI and hybridised with Ad5 DNA as described in Fig. 1 legend. The varying intensities of the bands in the different subclones from E11-L are due to uneven transfer of the DNA from the gel to the cellulose nitrate filter. The external B fragments migrated considerably more slowly than the corresponding marker B fragment. Note, however, that the external A fragments in transformed cell DNA did not appear to migrate more slowly than the corresponding A fragment of viral DNA because of the poor resolving power of the gel system in the region of the large A fragment (26.6 kilobase pairs). *b*, *Eco*RI restriction map of Ad5 DNA.

To determine whether or not the TPA enhancement of growth in agar was an irreversible effect, subclones of E11-L cells were isolated from agar lacking or containing TPA and further studied. Even in the absence of TPA these subclones grew with a 3.5- to 14.6-fold greater efficiency in agar than the parental clone (Table 1). Note that, with the exception of one subclone (E11-A-11), the addition of TPA to the agar resulted in a further enhancement (1.3- to 4.8-fold) in agar growth, and in some cases the cloning efficiency in agar than exceeded 50% (Table 1). Analysis of 14 additional E11-L subclones isolated from agar, which contained or lacked TPA, revealed that with serial passage in the absence of TPA none of these subclones reverted to the lower agar cloning efficiency characteristic of the parental E11-L clone. As with the parental E11-L clone, growth in agar of the subclones was not enhanced by 200 ng ml^{-1} of the non-tumour-promoting agents phorbol or 4αPDD.

When studied in monolayer culture, E11-L and all of its subclones had shorter average doubling times and higher saturation densities than normal 2°RE cells (Table 2). TPA did not significantly alter the average doubling time of normal 2°RE or any of the adenovirus-transformed clones (Table 2). TPA did, however, increase the saturation density of some, but not all of these cells. With serial passage E11 cells acquired a higher saturation density (compare E11-L to E11-E, Table 2) and, in general, the E11-L subclones derived from agar had higher saturation densities than the parental E11-L clone. However, there was no direct relationship between saturation densities and cloning efficiencies in agar of E11-L or its subclones, either in the absence or presence of TPA.

Previous studies have demonstrated that the transformation of Syrian hamster embryo cells following a single exposure to the carcinogen benzo(*a*)pyrene involves a series of progressive changes—morphological transformation, enhanced fibrinolytic activity, growth in agar and tumorigenicity[16,17]. These are acquired in a temporal fashion during repeated serial passage of the culture[16,17]. The acquisition of anchorage independence and tumorigenicity following the exposure of guinea pig embryo fibroblasts[18], rat brain cells[19], rat liver cells[20], or mouse epidermal cells[21], to a single exposure with chemical carcinogens has also been found to require extended serial passage (in these cases several months) in culture.

Table 1 Effect of TPA on anchorage-independent growth of normal and adenovirus-transformed rat embryo cells

Cell type	Colony number		Agar cloning efficiency (%)		TPA enhancement factor
	−TPA	+TPA	−TPA	+TPA	
2°RE	0	0	<0.001	<0.001	0
A18-E (<10 pass.)	0	2.4 ± 0.8	<0.001	0.1	>100
A18-L (>30 pass.)	2.8 ± 0.4	10.4 ± 2.2	0.1	0.5	5.0
E7-E (<10 pass.)	0	4.8 ± 2.0	<0.001	0.2	>200
E7-L (>30 pass.)	23.2 ± 2.4	54.4 ± 5.0	1.2	2.7	2.3
E11-E (<10 pass.)	22.2 ± 5.8	58.4 ± 6.4	1.1	2.9	2.6
E11-L (>30 pass.)	59.2 ± 17.8	232.0 ± 18.4	3.0	11.6	3.9
Subclones of E11-L isolated from agar					
E11 A-1 1h	252 ± 30.4	956 ± 76.8	12.6	47.8	3.8
E11 A-1 1i	204 ± 36.4	988 ± 59.2	10.2	49.4	4.8
E11 A-1 1l	424 ± 30.4	434 ± 40.6	21.3	21.7	1.0
Subclones of E11-L isolated from agar plus TPA					
E11 A-T-1 1b	826 ± 68.6	1,118 ± 72.4	43.1	55.9	1.3
E11 A-T-1 1u	232 ± 17.0	1,034 ± 72.2	11.6	51.7	4.5
E11 A-T-1 1y	245 ± 16.4	1,102 ± 96.6	12.7	55.1	4.3

Secondary rat embryo (2°RE) cultures were established from 14-d gestation Sprague–Dawley rat embryos[23] and were grown at 37 °C in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS). Clonal populations of type 5 adenovirus H5ts125-transformed 2°RE cells were formed as previously described[12] and were grown at 37 °C in 0.1 mM Ca^{2+} medium supplemented with 7.5% FBS (low-Ca^{2+} medium)[23]. Clones A18 and E7 were originally isolated from two foci (on different plates) of acetone pretreated (solvent control) H5ts125-transformed 2°RE cells and clone E11 cells were derived from a focus of benzo(*a*)pyrene (0.05 $\mu g\ ml^{-1}$)-pretreated, H5ts125-transformed 2°RE cells[12,13]. The original transformed cell population was cloned and tested for growth in agar followng early (E-, <10) and late (L-, >30) serial passage. E11-L subclones were isolated directly from agar lacking or containing 100 ng ml^{-1} TPA and were maintained as separate cell lines in low Ca^{2+} medium. Agar cloning efficiency of early (<10) and late (>30) passage adenovirus-transformed clones and E11-L subclones was determined by plating 2×10^3 cells in 4 ml of agar overlay medium containing 0.4% Noble agar prepared in low Ca^{2+} medium on top of prehardened 4-ml base layers consisting of 0.8% Noble agar in the same medium. Parallel cultures contained TPA at a final concentration of 100 ng ml^{-1} incorporated in both the agar overlay and base layers. Cultures were fed after 4 and 10 days with 3 ml of 0.4% Noble agar in low-Ca^{2+} medium containing or lacking 100 ng ml^{-1} TPA. Agar cloning efficiency of 2°RE cells in the presence or absence of 100 ng ml^{-1} TPA was determined as above except 2×10^4, 2×10^5 or 2×10^6 cells were seeded and the medium was DMEM in Noble agar. Colony number was determined by light microscopy after 14 days (colony size >0.1 mm) and 21 days (colony size >2 mm). Results reported are the average colony number from four replicate plates ± s.e.m. TPA enhancement factor refers to agar colony-forming ability in the presence of TPA relative to cultures not receiving TPA. Phorbol and 4α phorbol-12-13-didecanoate (4αPDD), two compounds inactive in tumour promotion, did not inhibit agar growth or induce enhanced agar cloning efficiency of any of the adenovirus-transformed clones even when tested at 200 ng ml^{-1}.

Experiments were therefore carried out to determine whether acquisition of anchorage independence following serial passage of adenovirus-transformed cells, or TPA treatment in agar, altered the amount or the pattern of viral DNA integration. Nucleic acid hybridisation techniques, reassociation kinetics[15], and Southern blotting filter hybridisation[22], were used to determine the amount of DNA present and the state of the DNA (that is, whether still integrated as single linear molecules). Reassociation kinetics indicated that E11-E and E11-L transformed cells each contained approximately one copy of the entire Ad5 genome. Furthermore, the Southern blotting technique revealed that the integration patterns of E11-E and E11-L were identical (data not shown) and that when the transformed cell DNA was cleaved with *Xba*I enzyme the integration patterns of viral DNA in E11-L and its subclones were indistinguishable (Fig. 1). Note that because of their attachment to host DNA, the terminal C and E fragments migrated more slowly than the C and E viral DNA marker fragments. Moreover, the C fragments of E11-L and all of its subclones co-migrated; similarly, all E fragments from E11-L and its subclones isolated from agar with or without TPA co-migrated with each other. In contrast, the internal fragments (A, B and D) from all clones co-migrated with the corresponding internal fragments of marker viral DNA, demonstrating that the viral DNA was integrated as intact linear molecules without interruption by cellular sequences and hence without disturbed specific endonuclease restriction sites. Similar conclusions can be drawn from patterns found following cleavage of transformed cell DNA with the *Eco*RI restriction endonuclease (Fig. 2).

The data presented here indicate that acquisition of anchorage-independent growth by adenovirus-transformed cells requires prolonged serial passage and that exposure to TPA markedly accelerates the acquisition of this property. These events are not due simply to random mutation and cell selection; their frequency is too high and they do not obey the properties of a classical mutation in a fluctuation analysis (P.B.F. and I.B.W., unpublished). In addition, the data presented indicate that these changes in cell phenotype are not due to alterations in the extent or state of integration of adenovirus sequences in the genome of the host cell. Two major alternatives remain to be considered: (1) a change in expression of the integrated viral sequences, either at the level of transcription and RNA processing or protein synthesis; or (2) alterations in the expression of specific host genes which might act in concert with integrated viral genes to modulate the phenotype of the transformants. We are now

Table 2 Effect of TPA on doubling time and saturation density of normal and adenovirus-transformed rat embryo cells

Cell type	Average population doubling		Saturation density (cells cm^2 × 10^5)	
	−TPA	+TPA	−TPA	+TPA
2°RE	32.2 ± 1.2	31.6 ± 1.5	1.7	2.1
A18-E (<10 pass.)	32.5 ± 1.0	29.5 ± 1.0	6.7	8.2
A18-L (>30 pass.)	19.4 ± 2.1	20.2 ± 1.7	8.7	9.9
E7-E (<10 pass.)	46.3 ± 3.5	45.7 ± 2.8	5.3	6.3
E7-L (>30 pass.)	21.4 ± 1.7	20.2 ± 2.1	7.9	9.5
E11-E (<10 pass.)	18.2 ± 1.0	15.4 ± 0.7	9.2	11.2
E11-L (>30 pass.)	15.2 ± 0.5	15.4 ± 0.7	12.8	14.8
Subclones of E11-L isolated from agar				
E11 A-1 1h	16.1 ± 0.8	14.2 ± 0.3	20.2	24.2
E11 A-1 1i	15.0 ± 0.5	15.5 ± 0.1	18.3	19.6
E11 A-1 1l	16.3 ± 0.4	16.5 ± 0.7	17.2	17.5
Subclones of E11-L isolated from agar plus TPA				
E11 A-T-1 1b	14.2 ± 0.6	14.6 ± 0.2	15.0	15.8
E11 A-T-1 1u	15.4 ± 0.7	13.0 ± 0.4	19.4	25.2
E11 A-T-1 1y	14.8 ± 0.8	15.9 ± 1.0	16.9	22.6

The average population doubling times and saturation densities of 2°RE and H5ts125-transformed clones in the presence or absence of TPA were obtained by plating 5 × 10^4 cells per 50 mm tissue culture plate in low-Ca^{2+} medium at 37 °C, changing the media after 6 or 24 h to low-Ca^{2+} medium with or without 100 ng ml^{-1} TPA and determining cell numbers over a 21-d period. Cells in triplicate plates were dispersed with trypsin/EDTA (0.25% trypsin w/v, 0.2% EDTA w/v) and counted every 24 h using a Coulter counter (Model Zf). The media were changed every 72 h with or without 100 ng ml^{-1} TPA. Population doubling times were calculated over a 72-h period corresponding to the logarithmic phase of growth. Saturation densities represent the maximum cell densities obtained in confluent cultures grown in the presence or absence of TPA.

analysing transcription patterns of viral mRNA in serially cultured clones of adenovirus-transformed cells, in the presence or absence of TPA, to determine whether changes in the type or amount of specific mRNA transcripts can be detected during the course of progression of these cell cultures. Thus, the cell system described here may be useful for understanding the mechanism by which transformed cells acquire anchorage-independent growth; it may also provide a useful model for studying the complex phenomena involved in tumour promotion and progression.

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Lysozyme-induced T-suppressor cells and antibodies have a predominant idiotype

Michael A. Harvey, Luciano Adorini, Alexander Miller & Eli E. Sercarz

Department of Microbiology, University of California, Los Angeles, California 90024

The existence of shared idiotypic determinants on the surfaces of T and B cells is now firmly established, suggesting that on both these cell types immunoglobulin variable regions are expressed which presumably function as antigen receptors. In most systems this has been inferred through the use of anti-idiotypic antibody instead of antigen to induce either helper or suppressor T cells[1,2]. Recent evidence demonstrates that antigen-specific suppressor or helper factors can also bear idiotypic determinants[3,4]. It is possible that these factors represent released receptors or portions of receptors. We show here the direct elimination of an antigen-induced T-suppressor population by an anti-idiotypic serum and complement. These suppressor T cells as well as the idiotypic population used to generate the antiserum are each specific for the same limited portion of the multi-determinant antigen, lysozyme. Apparently, these suppressor cells are restricted in specificity as well as share idiotypy with antibodies of the same specificity.

Lysozymes are small (molecular weight ~14,300) globular proteins. The amino acid sequence and three-dimensional structure for lysozymes from many different species are known[5]. The murine response to a series of bird egg white lysozymes, typified by chicken lysozyme (HEL), is under H-2-linked genetic control. Mice of the H-2^b and H-2^s haplotypes (B10 and B10.S) are non-responders while mice bearing other haplotypes are responders[6]. We have demonstrated T-suppressor cells in

Table 1 Anti-idiotype and complement treatment eliminates the activity of HEL-induced suppressor cells from B10 mice

Spleen cells (×10^6) per culture from mice primed with:		Treatment of HEL-primed spleen cells	Direct PFC per culture on day 4	
CFA	HEL–CFA		HEL	SRBC
2	—	—	231 ± 46	1,326 ± 37
—	2	—	65 ± 20	1,956 ± 449
1.8	0.2	—	53 ± 37	1,233 ± 203
1.8	0.2	NGPS + C′	31 ± 1	1,310 ± 70
1.8	0.2	Anti-id + C′	207 ± 10	1,233 ± 240
1.8	0.2	Anti-id	75 ± 5	1,533 ± 433

In vitro cell cultures were carried out using a miniaturised two-chamber diffusion culture system[14]. Culture medium was RPMI 1640 (GIBCO) supplemented with 10% fetal calf serum (Flow), 2 mM L-glutamine, 5 × 10^{-5} M 2-mercaptoethanol. B10 mice were primed intraperitoneally with CFA or HEL–CFA (100 μg per mouse) 4 weeks before culture. Spleen cells (2 × 10^6) were cultured together with 2 × 10^6 HEL-SRBC in 0.1 ml in the inner chamber, separated by a dialysis membrane from the reservoir in which 1.5 ml of medium was placed. At day 4 of culture, cells were collected and tested for direct PFC formation against HEL–burro (B)RBC, BRBC or sheep(S)RBC using the Cunningham and Szenberg technique[15]. Antiserum treatment: before culturing, 2 × 10^6 spleen cells were pelleted and resuspended in 0.1 ml of heat-inactivated normal guinea pig serum (NGPS) (final dilution 1 : 10) or guinea pig anti-idiotype (anti-id) (1 : 5) for 30 min at 4 °C. Cells were then washed once, resuspended in 0.1 ml rabbit complement (Cedarlane Low-Tox), diluted 1 : 8, incubated at 37 °C for 30 min and finally washed three times before culture. PFC data are presented as arithmetic means ± s.e.m. from three replicate cultures.

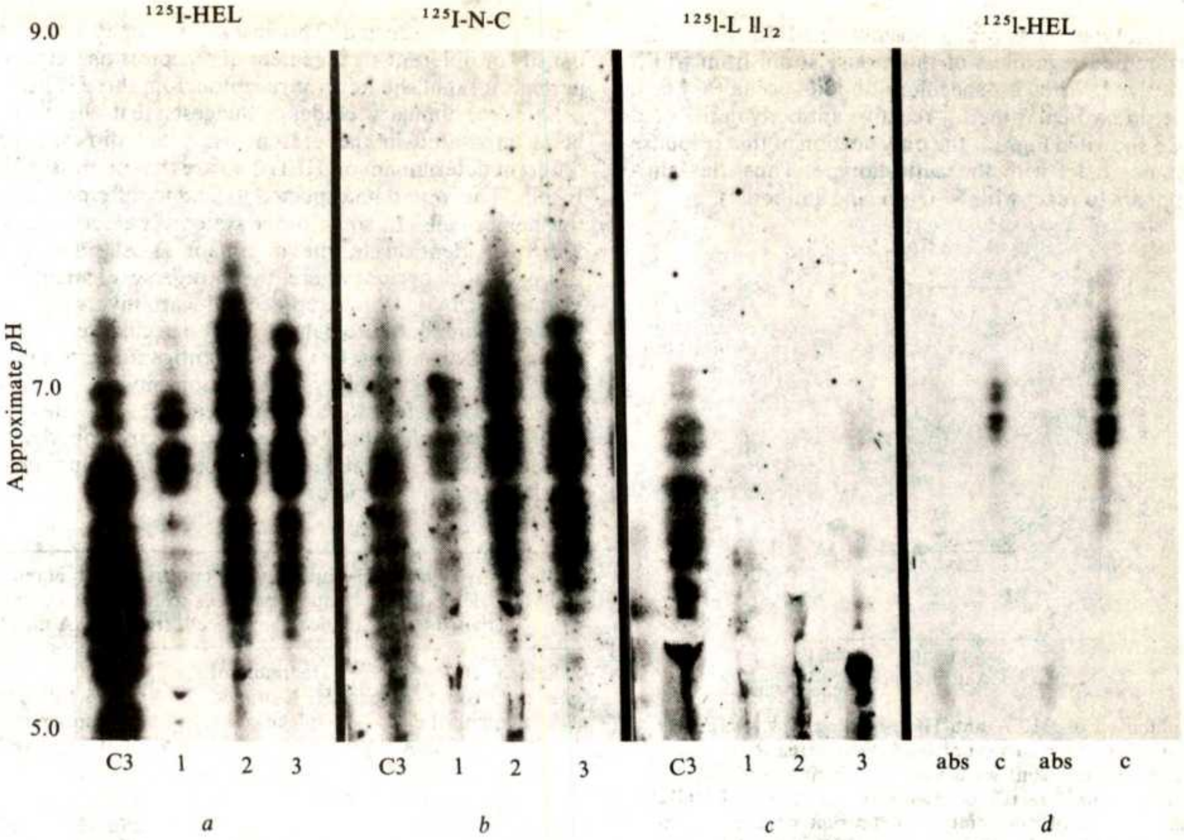

Fig. 1 Autoradiographs of isoelectric focusing in polyacrylamide gels showing specificity and idiotypy of anti-HEL antibodies. *a*, IEF patterns of 2 μl A/J C3 and B10.A (1, 2, 3) immune ascitic fluids, stained with ^{125}I-HEL. *b*, Same fluids as in *a* stained with ^{125}I-N–C peptide. *c*, Same fluids as in *a* stained with ^{125}I–L_{II} peptide. Isoelectric focusing was carried out as described previously[11]. Iodination of N–C peptide, which lacks tyrosine, was accomplished by first reacting the peptide with methyl-*p*-hydroxybenzimidate as described by Wood *et al.*[12], followed by iodination with the Chloramine T method[13], *d* Represents the effect of absorbing the anti-HEL from B10.A mice 1 and 3 with anti-idiotype before isoelectric focusing. Briefly, 2 μl of each B10.A anti-HEL was placed in a small conical centrifuge tube to which 10 μl guinea pig anti-idiotype was added. Control tubes were 2 μl ascitic fluid plus 10 μl normal guinea pig serum. All tubes were incubated for 30 min at 37 °C and overnight at 4 °C. The entire contents of each tube was then placed on isoelectric focusing gels. If immune complexes form during this incubation, specific antibody bands are eliminated from the autoradiograph, as these complexes appear to be too large to enter the gel. abs, absorption with anti-idiotype; C, the mixture with normal guinea pig serum. Left two lanes are from B10.A mouse 1; right two lanes are from B10.A mouse 3.

the spleens of B10 mice injected intraperitoneally with HEL emulsified in Freund's complete adjuvant (CFA). Helper cells are demonstrable in the spleens of similarly immunised congeneic B10.A mice[7]. We have also established that a disulphide-containing peptide derived from this multi-determinant antigen (N–C peptide = 1–17: Cys 6–Cys 127:120–129) is equally capable of inducing these suppressor cells while another peptide from HEL (L_{II} peptide = 13–105) induces helper T cells in B10 mice[8]. To further characterise T-cell receptors in this system we have examined the effect of anti-idiotype on HEL-induced suppressor cells from non-responding B10 mice and HEL-induced helper cells from responding B10.A mice.

To investigate the lysozyme T-cell idiotypic repertoire in B10.A and B10 mice, anti-idiotypic antisera were generated against restricted anti-HEL antibody populations, which are often found in B10.A mice. Figure 1*a* shows the isoelectric focusing patterns (IEF) of antibodies from immune ascitic fluid of three B10.A mice after immunisation with HEL. These patterns are more restricted than those produced by similarly immunised A/J mice.

Two lysozyme-derived peptides, N–C and L_{II}, were used to investigate the specificity of these anti-HEL antibodies. Figure 1*b* and *c* show the IEF patterns of the same fluids as in Fig. 1*a* stained with the N–C peptide and the L_{II} peptide, respectively. As can be seen, all the antibody in the ascitic fluid of the HEL-immunised B10.A mice appears to react with the N–C peptide but not with the L_{II} peptide. Experiments with N–C immunoadsorbents corroborate the N–C staining results (data not shown). The selected A/J fluid shows some L_{II} reactivity but it does not represent a large portion of the total antibody (~15%).

Purified antibody from the ascitic fluid of one B10.A mouse (1 of Fig. 1) was isolated by adsorption to and elution from an HEL immunoadsorbent. The isolated antibodies were used to immunise guinea pigs and the resulting antiserum repeatedly passed over normal B10.A Ig columns. Figure 1*d* shows that the absorbed serum reacts with all the antibody bands present in the immunising population as well as all other B10.A anti-HEL antisera. The addition of large amounts of normal Ig to absorption mixtures had no effect on the ability of the guinea pig anti-idiotype to absorb HEL-specific antibody bands (data not shown).

As idiotypic determinants are antigenic structures close to or including the antigen-binding site of the antibody molecule, anti-idiotypic antibodies may interfere with the interaction of idiotype with antigen. Figure 2 demonstrates that the anti-idiotypic serum can inhibit the binding of B10.A anti-HEL to radiolabelled HEL by as much as 80%. The same anti-idiotype has no effect on the binding of B10.A anti-human lysozyme (HUL) antibodies to radiolabelled HUL. This inhibition of HEL binding is consistent with recognition of determinants associated with, or near, the antigen-binding site by the anti-idiotypic serum.

This anti-idiotype reacts with almost all anti-HEL antibodies we have examined regardless of the mouse strain from which they were derived. When L_{II}-specific antibodies occur, they lack the idiotype; in particular the L_{II}-reactive antibody in the fluid from A/J C3 shown in Fig. 1 is the only portion of that response which does not react with the anti-idiotype. Thus, this anti-idiotype appears to react with N–C-specific antibodies.

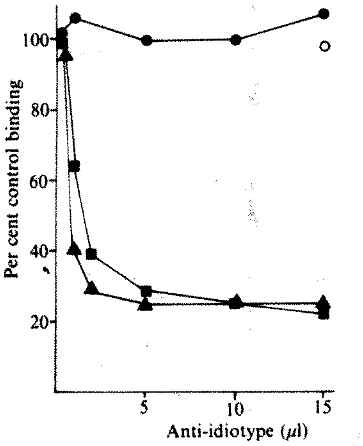

Fig. 2 Inhibition of B10.A anti-HEL binding to ^{125}I-HEL by anti-idiotype. Inhibition curves obtained when increasing amounts of anti-idiotypic antiserum were used to inhibit the binding of B10.A anti-HEL to ^{125}I-HEL or B10.A anti-HUL to ^{125}I-HUL. Increasing amounts of anti-idiotypic antiserum were added to tubes containing a suitable dilution of anti-HEL ascitic fluid. This dilution was capable of binding 49–55% of 10 ng of ^{125}I-HEL, or 10 ng of ^{125}I-HUL. Following appropriate incubation all the γ-globulin in each tube was precipitated by adding an equal volume of 36% (w/v) Na_2SO_4. After 2 h incubation at room temperature, the precipitates were collected by centrifugation, washed twice and counted in a Nuclear Chicago γ-counter. Data are presented as the per cent of control binding (that is, binding obtained in reaction mixtures without inhibitor). ■, B10.A mouse 1 anti-HEL; ▲, B10.A mouse 3 anti-HEL; ●, a single B10.A anti-HUL serum binding to ^{125}I-HUL. ○, Inhibition of B10.A mouse 1 anti-HEL binding to ^{125}I-HEL by 15 µl normal guinea pig serum.

This anti-idiotypic serum was used to examine the expression of idiotypic determinants on functional T-cell subpopulations. Results in Table 1 demonstrate that preimmunisation of B10 mice with HEL generates a cell population capable of suppressing the anti-HEL plaque-forming cell (PFC) response of primary spleen cell cultures stimulated *in vitro* with HEL–red blood cell (RBC). These suppressor cells have been shown to be sensitive to rabbit anti-mouse thymocyte or anti-I-J treatment[7]. Furthermore, suppressive activity is found in the cell population remaining after passage through nylon wool (B. Araneo, personal communication). Sequential treatment of HEL-primed spleen cells with anti-idiotype and complement eliminates the ability of these cells to suppress the anti-HEL PFC response, while treatment with normal guinea pig serum and complement does not affect suppression. If complement is omitted and the cells are only incubated with anti-idiotype for 30 min at 4 °C, attempting to block suppressive activity, almost no effect is observed. These results indicate that the suppressor T cells raised against HEL express some or all of this set of idiotypic determinants.

Using the same approach we tried to determine if these idiotypes were expressed on helper T cells. Results shown in Table 2 demonstrate that the helper activity induced in B10.A mice by HEL priming is not affected by treatment of HEL-primed spleen cells with anti-idiotype and complement, while in the same experiment, HEL-induced suppressor cells in B10 mice are sensitive to this treatment. Even when sodium azide (10^{-2} M) was added to stabilise membrane receptors, the helper activity was unaffected. This insensitivity may reflect a lower density or different arrangement of receptors on helper cells, or perhaps a rapid shedding of receptor from these cells.

Some preliminary evidence suggests that the helper cells being measured in the present assay are directed against a different determinant on HEL than are the suppressor T cells or B cells. This would be expected to lead to different idiotypy for the helper cells. In some other systems, helper cells can bear idiotypes identical to the dominant B-cell idiotype. Interestingly, this occurs where the antigenic element is multivalent, such as with streptococcal carbohydrates[9] or phosphorylcholine[10], where helper T cells specific for the repeating determinant can present the same antigenic determinant to B cells. In our system, this type of relationship is obviated because HEL is monovalent for any given epitope. The clear requirement for helper T cells of different specificity from that of the B cell could quite naturally lead to a functional helper population lacking the dominant idiotype.

Table 2 Anti-idiotype and complement treatment eliminates the activity of HEL-induced suppressor cells from B10 mice but not the activity of HEL-induced helper cells from B10.A mice*

Spleen cells (×10^6) per culture from mice primed with:		Treatment of HEL-primed spleen cells	Direct PFC per culture on day 4	
CFA	HEL–CFA		B10	B10.A
2	—	—	170±27	26± 1†
—	2	—	11± 7	235±59
1.9	0.1	—	25± 2	155± 5
1.9	0.1	NGPS+C′	31±13	150±20
1.9	0.1	Anti-id+C′	186±31	161±29

* Immunisations, *in vitro* cultures, and antisera treatments as described in Table 1. HEL–human RBC were substituted for HEL–SRBC as antigen *in vitro*.

† Primary anti-HEL PFC responses obtained with normal B10.A spleen cells are routinely much lower than those obtained with normal B10 spleen cells.

Thus, the antibody specificity in the secondary response to lysozyme, a multi-determinant antigen, is restricted. Likewise, suppressor cells raised in the non-responder mouse show a remarkably similar restriction to this same determinant. We consider it no accident that receptors on each of these two cell populations should bear the same idiotypic determinants. This arrangement ensures that there will be mediation between suppressor T cells and B cells by helper T cells with complementary idiotypic or epitypic receptors. We are now studying the rules of antigenic and idiotypic circuitry which are involved in the selection of particular B cells for activation.

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Immunoreactive forms of human erythrocyte ankyrin are present in diverse cells and tissues

Vann Bennett

Department of Molecular Biology, Wellcome Research Laboratories, Research Triangle Park, North Carolina 27709

Ankyrin is a polypeptide of molecular weight (MW) 200,000 which is tightly bound to the cytoplasmic surface of the human erythrocyte membrane and has been identified as the high-affinity membrane attachment protein for spectrin[1-3]. This protein has also been shown to be associated with band 3 (ref. 4), the major transmembrane protein in erythrocytes. Ankyrin is thus an example of a polypeptide which links a cytoplasmic structural protein to an integral membrane protein. A water-soluble, 72,000-MW, proteolytic fragment of ankyrin has been purified which retains the ability to bind to spectrin, and competitively inhibits reassociation of spectrin with membranes[5]. Monospecific antibodies directed against this fragment have been prepared[1] and demonstrated to cross-react only with ankyrin among the erythrocyte membrane proteins[1,4]. The present study reports the use of these antibodies to develop a radioimmunoassay capable of detecting femtomolar quantities of ankyrin, and demonstrates the presence of small but significant amounts of immunoreactivity in a variety of types of cells and tissues.

Ankyrin itself is not suitable as a ligand for radioimmunoassay because it is very sensitive to proteolysis, and has hydrophobic properties which would make nonspecific binding a serious problem[1]. The 72,000-MW water-soluble fragment of ankyrin, however, was readily radioiodinated and exhibited low background radioactivity in assay conditions. Monospecific anti-fragment IgG bound ^{125}I-labelled fragment with both a high-affinity interaction ($K_D = 0.3$ nM) and a lower-affinity association ($K_D = 12$ nM); 75–80% of the ^{125}I-labelled fragment was immunoreactive.

Binding of ^{125}I-labelled fragment to low concentrations of monospecific antibody (0.2 nM) was blocked completely by 0.4 pmol of unlabelled fragment, and a significant inhibition occurred with as little as 2 fmol of fragment (Fig. 1). Binding assays were carried out in the presence of Triton X-100, which permitted analysis of membranes and whole cells without prior solubilisation. Human erythrocyte ghosts block binding of ^{125}I-labelled fragment completely above 500 ng of membrane protein, and by 50% at 20 ng of protein (Fig. 1).

To estimate the amount of ankyrin present in ghost membranes, it was necessary to control for the possibility that the protein in a native conformation might associate with antibody to a different degree from its association with the soluble fragment. Accordingly, SDS-denatured fragment and ghost membranes were assayed for inhibition of binding. SDS-treated fragment exhibited unchanged activity, whereas SDS-treated membranes were twofold less effective (Fig. 1). The SDS-treated ghosts contained the equivalent of 0.36 pmol or 26 ng of fragment per μg membrane protein, which agrees closely with the previous estimate that the fragment constitutes 2.4% of the membrane protein[5]. Ankyrin thus comprises 7.2% of the total protein, a value corresponding to about 120,000 molecules per erythrocyte using the data of Steck for erythrocyte membrane proteins[9]. (This value is based on the assumption that the 72,000-MW fragment of ankyrin does not contain antigenic determinants that are repeated in the remainder of the molecule.)

Radioimmunoassay provides quantitative information only when the ligand used as a standard and the material to be assayed bind to the antibody with the same affinity and the same extent of cross-reaction. However, useful qualitative comparisons of levels of immunoreactivity can be made in heterologous systems, although the actual amount of antigen will in most cases be underestimated. Peripheral human blood neutrophils, platelets and human cultured fibroblasts from foreskin or embryonic lung exhibited a significant amount of immunoreactivity (Fig. 2). ^{125}I-labelled fragment was displaced by 50% using 5×10^4 erythrocytes, 8×10^3 neutrophils, 2×10^6 platelets and 1.4×10^5 fibroblasts per assay. These numbers correspond to 6×10^5 copies of ankyrin per neutrophil, 3×10^3 per platelet and about 3×10^4 per fibroblast. The slopes of the inhibition curves of these cells are less than that of erythrocytes, indicating partial cross-reactivity and/or decreased affinity for the antibody. The values for molecules per cell are thus probably underestimates. The activity of these cells is unlikely to be due to proteolysis by lysosomal enzymes, as samples boiled in SDS still compete for binding. Furthermore, problems of nonspecific interference in the assay were minimised by the very small quantities of cells used.

It was important to determine whether immunoreactivity could be detected in various tissues. Due to difficulty in obtaining fresh human material, erythrocytes from other species were compared for cross-reactivity. Rat cells were about 10-fold less active than those of humans, but were 3–5-fold more reactive than cells of horses or rabbits. Membrane fractions from rat brain, liver, kidney, testes and isolated epidydimal fat cells all exhibited immunoreactivity, with 50% inhibition at 0.4 μg for rat erythrocytes, 5.7 μg for brain, 9.5 μg for testes and about 12.6 μg for liver, kidney and fat cells (Fig. 3). These values correspond to the presence of ankyrin at 0.5% of the total membrane protein in brain, 0.2% in liver, kidney and fat cells, and 0.3% in testes, assuming that rat and human erythrocytes

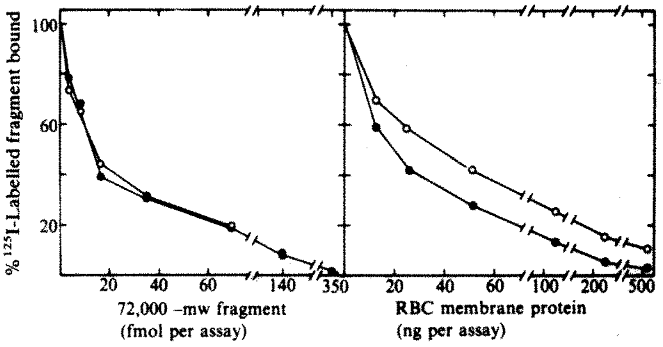

Fig. 1 Inhibition of binding of ^{125}I-labelled 72,000-MW fragment to monospecific anti-fragment IgG by unlabelled 72,000-MW fragment (left) and human erythrocyte membrane protein (right). Anti-fragment IgG (0.18 nM) was incubated for 1 h at 0 °C in 0.075 ml of buffer (100 mM NaCl, 10 mM sodium phosphate, *p*H 7.5, 1 mM EDTA, 250 μg ml^{-1} bovine serum albumin, 5 μg ml^{-1} pancreatic trypsin inhibitor, 0.2% (v/v) Triton X-100, 10 μg ml^{-1} preimmune IgG) in the presence of various concentrations of 72,000-MW fragment or erythrocyte ghost protein which were either untreated (●) or had been exposed to SDS (0.1%) (○). The final concentration of SDS was not more than 0.0025% at the highest dilution of these samples. ^{125}I-labelled fragment (prepared by the method of Hunter and Greenwood[6]; 3.6×10^6 c.p.m. pmol^{-1}, 0.4 nM final concentration) and 0.2 μl protein A-bearing staphylococci were then added as a second antibody[7], and the incubation was continued for another hour at 0 °C. The reaction was terminated by addition of 3.5 ml 2 M urea, 0.1 M glycine and 1% (v/v) Triton-X-100, and the bacteria, with adsorbed IgG, were collected by centrifugation (10 min, 3,000*g*), and analysed for ^{125}I. The data are presented as per cent of binding in the absence of additions, and are corrected for nonspecific binding by subtracting the value obtained with preimmune IgG alone at each concentration of fragment and membrane protein. Erythrocyte ghosts were prepared by hypotonic lysis in 7.5 mM sodium phosphate, *p*H 7.5 (ref. 8).

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contain the same amount of ankyrin and that the immunoreactive protein has the same molecular weight as ankyrin. Liver homogenates were fractionated by differential centrifugation into microsomal membranes which contained 55% of the immunoreactivity, a mitochondrial/lysosomal pellet which had 7% and a soluble fraction which had 30%. The soluble immunoreactivity may be due to proteolytic cleavage of the membrane-bound protein, for the 72,000-MW fragment of erythrocyte ankyrin is released frŏm the membrane with very limited proteolysis[5].

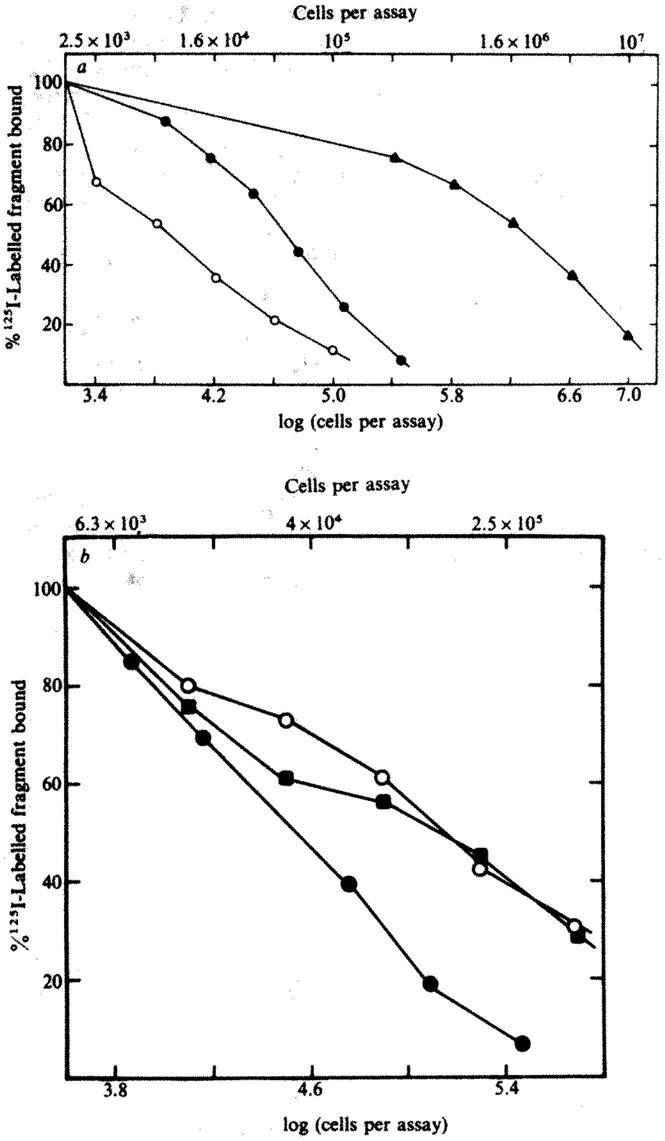

Fig. 2 Inhibition of binding of ^{125}I-labelled 72,000-MW fragment to monospecific anti-fragment IgG by human erythrocytes, neutrophils and platelets (a), and by cultured human foreskin fibroblasts and Wi-38 lung fibroblasts (*b*). Anti-fragment IgG (0.18 nM) was incubated as described in Fig. 1 in the presence of various amounts of human erythrocytes (●), neutrophils (○) and platelets (▲) (*a*), and, in a separate experiment, with human erythrocytes (●), cultured foreskin (■) and Wi-38 lung (○) fibroblasts. Binding of ^{125}I-labelled 72,000-MW fragment (3.6 × 10^6 c.p.m. pmol^{-1}, 0.6 nM) was then determined (Fig. 1). Peripheral blood cells were separated into erythrocytes, neutrophils and a combined lymphocyte and platelet fraction by the method of Boyum[10]. Lymphocytes and platelets were separated by sedimentation on sucrose gradients[11]. Cultured human fibroblasts from a foreskin explant and from embryonic lung (Wi-38) were grown to confluency in Eagle's minimum essential medium supplemented with 10% (v/v) fetal calf serum, and were detached after exposure to 150 mM NaCl and 5 mM Na EDTA, *p*H 7.5. The erythrocytes in these assays contain the equivalent of 100,000 ankyrin molecules per cell based on a radioimmunoassay with SDA-denatured cells and comparison with a standard curve of 72,000-MW fragment.

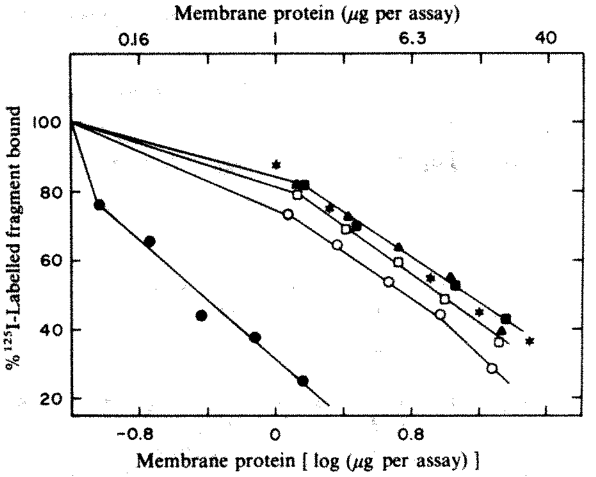

Fig. 3 Inhibition of binding of ^{125}I-labelled 72,000-MW fragment to monospecific anti-fragment IgG by membrane protein from rat erythrocyte ghosts, brain, liver, kidney, testes and epidydimal fat cells. Anti-fragment IgG (0.23 nM) was incubated in the presence of various concentrations of membrane protein from rat erythrocyte ghosts (●), brain (○), liver (■), kidney (▲), testes (□) and epidydimal fat cells (*), and binding of ^{125}I-labelled fragment (2 × 10^6 c.p.m. pmol^{-1}, 0.5 nM) was then determined (Fig. 1). Membrane fractions from rat tissues were prepared by homogenising 1 g of perfused liver or kidney, brain cerebrum, testes or isolated fat cells from eight 100-g rats[12] in 9 ml of 0.25 M sucrose, 2 mM Na EGTA, *p*H 7.0, and 200 μg ml^{-1} phenylmethylsulphonyl fluoride with a Brinkman polytron (30 s, 3,200 r.p.m.); the suspensions were pelleted for 10 min at 900*g* to remove nuclei, large particles and possible contaminating erythrocytes. The supernatants were then diluted 10-fold and centrifuged for 30 min at 250,000*g* to collect the membrane fractions.

The possibility of contamination of the membrane fractions from solid tissues by erythrocytes was minimised by using conditions for homogenising the tissues that lyse only 10–15% of erythrocytes, and including a low-speed centrifugation step to remove unbroken cells before isolation of the membranes. The amount of contamination by intact and lysed erythrocytes in these low-speed supernatants was estimated by solubilising membranes with Triton X-100, and measuring haemoglobin by absorbance at 578 nm. The values obtained by this assay corresponded to a maximum contamination of membranes with erythrocyte ankyrin of 0.005% for brain, 0.009% for liver, 0.008% for kidney and 0.006% for testes. The activity of these membrane fractions thus cannot be explained by contamination with erythrocyte membranes. Furthermore, fat cells have a density of less than 1 g cm^{-3}, and consequently float whereas other cells are sedimented. These buoyant cells are thus completely free of erythrocyte contamination, and still contain the same amount of immunoreactivity as liver and kidney.

The small amounts of immunoreactivity in these membrane fractions, and the extreme sensitivity of ankyrin to proteolysis, makes a detailed analysis of the cross-reacting polypeptides quite difficult. However, it was possible to immunoprecipitate with anti-ankyrin IgG a polypeptide of MW ~200,000 from ^{125}I-labelled brain and fat cell membranes (not shown). This polypeptide was not precipitated in the presence of excess, 72,000-MW ankyrin fragment, nor by preimmune IgG. Many other polypeptides were also present in the immunoprecipitates, but their precipitation was not reduced by the ankyrin fragment, and they were also obtained with preimmune IgG.

The molecular weight of erythrocyte ankyrin is similar to that of myosin and of filamin. These proteins, however, did not contribute to the cross-reactivity in these various cell types. as partially purified myosin or filamin from human platelets were not active in the radioimmunoassay. Furthermore, the total protein fraction from rat skeletal myofibrils exhibited very low activity.

These results demonstrate the ubiquitous presence of small amounts of an ankyrin-like protein distinct from myosin or filamin which is associated primarily with membrane components. Ankyrin links band 3 to the erythrocyte cytoskeleton[4], and may have a related function in specialised regions of the plasma membrane in other cells. Spectrin was not detected in cultured cells by radioimmunoassay[14], but this negative result could be explained by poor cross-reactivity of the antibody or by insufficient sensitivity of the assay. It is conceivable that proteins analogous to ankyrin, spectrin and band 3 could be present together in non-erythrocyte cells, and could be involved in processes requiring direct contact between integral membrane proteins and structural proteins on the inner surface of the membrane.

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A mutation in *Caenorhabditis elegans* that increases recombination frequency more than threefold

A. M. Rose & D. L. Baillie

Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia, Canada

In higher organisms the rate of recombination between genetic loci is presumably responsive to selective pressure. Recently, selective pressures[1] and mutational events[2] that influence recombination have been reviewed. Mutational sites and chromosomal rearrangements that enhance or suppress recombination frequency in specific regions are known, but general mechanisms that enhance recombination have not yet been discovered. We describe here the isolation and characterisation of a strain of the hermaphroditic nematode, *Caenorhabditis elegans*, that has a recombination frequency at least threefold higher than that found in the wild type[3,4]. In this strain, *rec-1*, the number of reciprocal recombination events between linked loci is increased. This is true for all pairs of linked loci studied so far. The high recombination strain behaves as if it carries a classical, recessive mutation, although a second mutation exists which can alter the recessive behaviour of *rec-1*.

We established a strain heterozygous for mutations in closely linked loci on chromosome I. This strain, *dpy-5 unc-15+/++unc-13*, has a wild-type appearance. When allowed to self-cross it segregates dumpy paralysed (*dpy-5 unc-15/dpy-5 unc-15*), uncoordinated (*unc-13/unc-13*) and wild-type appearing (*dpy-5 unc-15+/++unc-13*) progeny. *dpy-5 unc-15/dpy-5 unc-15* are short, fat worms that cannot move, and *unc-13/unc-13* have a characteristic contorted appearance and movement. In addition to these phenotypes, recombinant dumpy (*dpy-5+/dpy-5 unc-15*) and paralysed (*+unc-*15*/dpy-5 unc-15*) progeny will occur. By selecting the hermaphrodites with wild-type appearance, this strain was maintained for several generations, and eventually scored for recombination between the *dpy-5* and *unc-15* loci. The frequency of recombination in this region was known from previous experiments[4] to be approximately 2% at 20 °C when all the progeny of a single parent were scored. In the heterozygous strain that we were maintaining, the recombination frequency between *dpy-5* and *unc-15* was approximately 7%. We hypothesised that our *unc-13/unc-13* strain, used to make the heterozygous strain, carried a factor responsible for this increase in recombination frequency. To demonstrate the existence of this factor, we out-crossed our uncoordinated strain to wild type and generated *unc-13/+* males. These males were crossed to *dpy-5 unc-15/dpy-5 unc-15*. On self-crossing, half the F_1 heterozygotes from this cross showed a high frequency of recombination among their progeny, while the other half had a normal recombination frequency. Successive generations of the high recombination individuals were measured for recombination frequency until a homozygous strain of high recombination hermaphrodites (XX) was established. This strain was treated at 26 °C for 30 h to produce males (X0) by X-chromosome nondisjunction. When these males were crossed to *dpy-5 unc-15/dpy-5 unc-15*, all the F_1 heterozygotes segregated a high number of recombinant individuals. After elimination of *dpy-5*, *unc-15* and *unc-13* mutant alleles by recombination, we established a male strain homozygous for *rec-1*, but having no visible mutant phenotype.

Table 1 The effect of *rec-1* on recombination frequency (%)

	Parental genotypes		
ab	*ab/++*	*ab/++; rec-1/+*	*ab/++; rec-1/rec-1*
dpy-5 unc-13 (I)	1.51±0.3*	1.30±0.3	12.41±1.6
dpy-14 unc-56 (I)	†	0.61±0.2	4.14±1.5
unc-22 unc-43 (IV)	1.90±0.6	1.55±0.4	3.95±1.4
dpy-11 unc-42 (V)	2.21±0.3	2.60±0.7	7.92±1.8

* ±95% confidence intervals.
† Not determined.

Table 2 The influence of *s155* on the expression of *rec-1*

	Parental genotypes	
ab	*ab/++; s155/+*	*ab/++; s155/+ rec-1/+*
	Recombination (%)	
dpy-5 unc-13 (I)	1.15±0.3	7.34±0.9
dpy-5 unc-15 (I)	1.80±0.4	9.11±0.9
dpy-5 dpy-14 (I)	1.62±0.6	5.50±0.9

Males from this strain were crossed to *dpy-5 unc-13/dpy-5 unc-13* individuals. Unlike before, all the F_1 heterozygotes produced a normal number of recombinants among their progeny. In the F_2, two types of heterozygote occurred—those which gave a normal frequency of recombinants and those which gave a high frequency of recombinants. Approximately one-quarter of the F_2 heterozygotes had high recombination frequencies and produced true-breeding high recombination progeny. In a parallel control experiment, *dpy-5 unc-13/dpy-5 unc-13* were crossed to *+/+* males and scored for recombination frequency in the F_1, F_2 and F_3. In this case the recombination frequency remained approximately 2% in every generation. From these studies, we concluded that the *rec-1* strain has a segregating factor which assorts independently of chromosome I. This factor is responsible for at least a threefold elevation in recombination frequency.

To investigate the generality of this increased recombination phenomenon, *rec-1/rec-1* males were crossed to other pairs of linked loci on chromosomes I, IV and V. The results showed that *rec-1* acts recessively to increase recombination frequency between two pairs of linked loci on chromosome I (Table 1). On

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chromosomes IV and V, recombination frequency in the *unc-22 unc-43* and the *dpy-11 unc-42* regions, respectively, was measured. Here also, an increase in recombination frequency was observed (Table 1). We interpret these results as evidence that *rec-1* is a general enhancer of recombination frequency in *C. elegans.*

With certain pairs of linked loci, a high recombination frequency was observed in the F_1 generation. For example, when *rec-1* males were crossed to *dpy-5 unc-15/dpy-5 unc-15* or *dpy-5 dpy-14/dpy-5 dpy-14*, the F_1 heterozygotes had a higher recombination frequency than wild type. From these results we hypothesised that *dpy-5 unc-15/++* and *dpy-5 dpy-14/++* individuals carry a site, *s155*, responsible for the dominant behaviour of the high recombination factor.

A strain, homozygous for *dpy-5 unc-13* and *s155* was constructed. If *s155* is, in fact, responsible for conferring dominant behaviour on *rec-1*, then *s155*-carrying *dpy-5 unc-13/dpy-5 unc-13* individuals should behave differently from *dpy-5 unc-13/dpy-5 unc-13* individuals when crossed to *rec-1*. Table 2 shows that when pairs of loci on chromosome I are tested in the presence of *s155*, *rec-1* acts dominantly. This provides evidence that a second mutation can modify the recessive nature of *rec-1*. Experiments are in progress to test whether *s155* can confer dominance on regions on other chromosomes.

We believe this to be the first demonstration of a general enhancer of recombination frequency in a higher eukaryote. The existence of a gene that can alter recombination and that is susceptible to mutation provides a mechanism whereby recombination frequency can be selected. It may be possible that, in addition to this allele of *rec-1*, other alleles which decrease recombination frequency can be recovered. If so, perhaps different levels of recombination frequency are selected in exclusively self-crossing or exclusively out-crossing populations. Furthermore, our *rec-1* strain may have an elevated spontaneous mutation rate. Three new visible phenotypes independent of *rec-1* have been observed in this strain. Thus, it is possible that the mechanism by which *rec-1* acts also alters the mutation frequency. This and other questions regarding the mode of action of *rec-1* are being investigated.

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Correlation between fragmented immunoglobulin genes and heavy chain deletion mutants

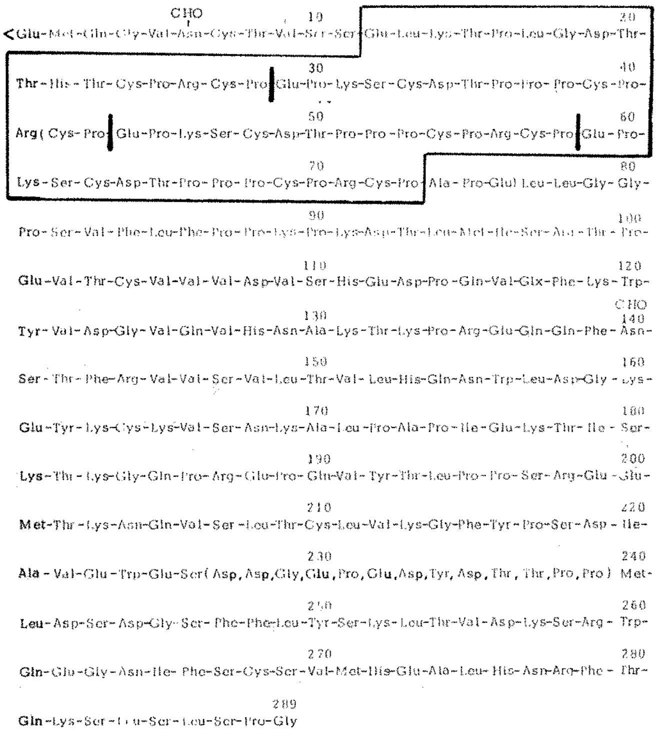
Blas Frangione* & Edward C. Franklin

Irvington House Institute, Departments of Pathology* and Medicine, New York University Medical Center, 550 First Avenue, New York, New York, 10016

It is generally accepted that the variable (V) and constant (C) regions of immunoglobulin (Ig) chains are under separate genetic control[1]. The notion that the different domains and interdomain regions are also under the control of independent genetic units was initially based on the clearcut results obtained by studying the primary structure of deletion mutants[2-9] and received definitive support from direct analysis of cloned heavy (H) and light (L) chain genes[10-13]. Here we present additional studies carried out on two selected γ3 deletion mutants which indicate that the genetic control of human H chains may be even more complex than previously believed.

L and H chains of Ig molecules consist of two functionally differentiated segments: the amino-terminal V region which contains the antigen binding sites, and the carboxy-terminal C region[14]. Sequence and crystallographic studies have shown that the C region of the IgG H chain is composed of three domains: CH1, CH2 and CH3. The striking structural homology among the C region domains, each of which is composed of about 110 residues folded into an almost identical tertiary structure, and the lesser homology to the V regions suggest a common evolutionary origin by a series of gene duplications. Each domain has a well defined function. The CH1 domain contains the attachment point for the L chain and pairs with the CL domain; together with paired VH and VL domains containing the antigen binding sites they form the Fab fragments of the IgG molecules. The CH2 domains, which act in complement fixation, and the CH3 domains, which are involved in cell surface interactions together form the Fc fragment. In the middle of the H chains of all subclasses of IgG, there is a region of 15–62 residues of unknown origin and function called the hinge region or Fh fragment, which separates the Fab and Fc fragments[15,16]. Thus, the γ H chain is composed of five discrete structural and functional units.

Heavy chain disease (HCD) protein SPA is an 80,000-molecular weight (MW) dimeric γ3 human H chain mutant with a blocked amino terminus. The amino-terminal sequence after unblocking is: $<\overset{1}{\text{Glu}}$-Glu-Glu-Val-Arg-Glu-Ser-$\overset{8}{\text{Glu}}$-Leu-Lys-Thr-Pro-Leu-Gly-Asp-Thr-Thr-(His)-Thr-Cys-Pro-(Pro)-Cys-Pro. Comparison with the prototype VH amino-terminal sequences shows that although the first seven residues bear some resemblance to the normal amino terminus they do not correspond to any known VH subgroup or CH1 sequence[25]. Position 8 is the beginning of the normal γ3 hinge region[16]. On the basis of its amino acid analysis and high cysteine content it seems



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30 40
Thr-His-Thr-Cys-Pro-Arg-Cys-Pro-Glu-Pro-Lys-Ser-Cys-Asp-Thr-Pro-Pro-Pro-Cys-Pro-
50 60
Arg(Cys-Pro-Glu-Pro-Lys-Ser-Cys-Asp-Thr-Pro-Pro-Pro-Cys-Pro-Arg-Cys-Pro-Glu-Pro-
70 80
Lys-Ser-Cys-Asp-Thr-Pro-Pro-Pro-Cys-Pro-Arg-Cys-Pro-Ala-Pro-Glu) Leu-Leu-Gly-Gly-
90 100
Pro-Ser-Val-Phe-Leu-Phe-Pro-Pro-Lys-Pro-Lys-Asp-Thr-Leu-Met-Ile-Ser-Arg-Thr-Pro-
110 120
Glu-Val-Thr-Cys-Val-Val-Val-Asp-Val-Ser-His-Glu-Asp-Pro-Gln-Val-Glx-Phe-Lys-Trp-
130 CHO 140
Tyr-Val-Asp-Gly-Val-Gln-Val-His-Asn-Ala-Lys-Thr-Lys-Pro-Arg-Glu-Gln-Gln-Phe-Asn-
150 160
Ser-Thr-Phe-Arg-Val-Val-Ser-Val-Leu-Thr-Val-Leu-His-Gln-Asn-Trp-Leu-Asp-Gly-Lys-
170 180
Glu-Tyr-Lys-Cys-Lys-Val-Ser-Asn-Lys-Ala-Leu-Pro-Ala-Pro-Ile-Glu-Lys-Thr-Ile-Ser-
190 200
Lys-Thr-Lys-Gly-Gln-Pro-Arg-Glu-Pro-Gln-Val-Tyr-Thr-Leu-Pro-Pro-Ser-Arg-Glu-Glu-
210 220
Met-Thr-Lys-Asn-Gln-Val-Ser-Leu-Thr-Cys-Leu-Val-Lys-Gly-Phe-Tyr-Pro-Ser-Asp-Ile-
230 240
Ala-Val-Glu-Trp-Glu-Ser(Asp, Asp, Gly, Glu, Pro, Glu, Asp, Tyr, Asp, Thr, Thr, Pro, Pro) Met-
250 260
Leu-Asp-Ser-Asp-Gly-Ser-Phe-Phe-Leu-Tyr-Ser-Lys-Leu-Thr-Val-Asp-Lys-Ser-Arg-Trp-
270 280
Gln-Glu-Gly-Asn-Ile-Phe-Ser-Cys-Ser-Val-Met-His-Glu-Ala-Leu-His-Asn-Arg-Phe-Thr-
289
Gln-Lys-Ser-Leu-Ser-Leu-Ser-Pro-Gly

Fig. 1 The amino acid sequence of human γ3 heavy chain deletion mutant WIS. Position 1 to 7 is an unusual V sequence; position 8 to 11 corresponds to VC or junction sequence; position 12 is the beginning of the hinge region of normal γ3 heavy chain which is 62 amino acid residues long (inside box) and four homologous subunits indicated by vertical bars. The first is a 17-residue segment followed by a 15-residue segment which is identically and consecutively repeated three times. CHO: carbohydrate; <Glu: 5-pyrrolidone-2-carboxylic acid.

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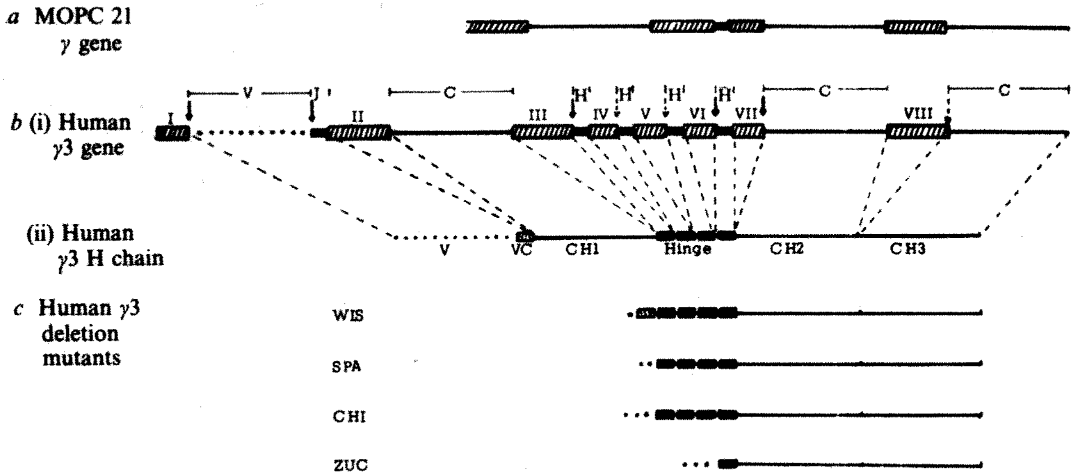

Fig. 2 Organisation of immunoglobulin H chain genes. Correlation with γ3 chain and γ3 deletion mutants. *a*, Arrangement of genes coding for MOPC 21 γ1 H chain constant region[13]. *b*(i), Proposed arrangement for human γ3 genes: . . . , coding DNA for V region; ——, coding DNA for C domains; ▩, coding DNA for J junction of VC region; ■, coding DNA for hinge or H junction region; ▨, (IVS) intervening sequences. IVS II, III, VII and VIII are present in the cloned DNA fragment coding for the murine γ1 constant region[13], and IVS I–VII are suggested by the structure of γ3 deletion mutants and myeloma proteins with a deleted hinge[5,23,24]. ↓, Indicate splicing sites as defined by deletion mutants; ⇣, not observed in man. *b*(ii), Human γ3 heavy chain. . . . V domain; ▩, VC region; ■, hinge region (quadruplicated); ——, CH1, CH2, CH3: constant domains. *c*, γ3 deletion mutants. Heavy chain disease proteins WIS, SPA, CHI[7] and ZUC[2,17]. For a complete list of deleted H chains see refs 6 and 9 for mouse and human, respectively.

likely that the intact hinge and the entire Fc fragment are present and that protein SPA has an internal deletion of about 210 residues comprising most of the V, the VC or J (junction) and CH1 regions.

HCD protein WIS is an 86,000-MW protein composed of two identical 289-residue chains. Figure 1 shows its amino acid sequence (details will be published elsewhere). It has two carbohydrate moieties attached to Asn 6 and Asn 140 and position 7 contains an unusual cysteine residue. Homology of the first 11 residues to both VH and the VC junction region has been previously pointed out[8]. The size of the deletion is about the same as that of protein SPA as position 12 marks the beginning of a normal hinge, consisting of 62 residues composed of 4 homologous regions. The hinge starts with a 17-residue segment (residues 12–28) followed by a 15-residue segment which is identically and consecutively repeated three times (residues 29–43, 44–58 and 59–73). The sequence from residue 74 to the carboxy terminus seems to be similar to that of previously reported Fc fragments[17].

The unexpectedly high frequency of deletion mutants belonging to the γ3 subclass allows conclusions of general significance. Figure 2*c* compares protein WIS and SPA with two other previously reported γ3 deletion mutants, protein CHI (ref. 7) and ZUC (refs 2, 17). The most constant feature of γ-chain deletion mutants is the presence of an internal deletion involving part of the VH and usually all of the CH1 domains which usually ends at the hinge. In the case of γ3 mutants, the deletion can end either at the beginning of quadruplicated hinge, or in one case (ZUC), at the beginning of one of the segments. In contrast, in different γ heavy chain deletion mutants the amino terminus can be normal—similar but not identical to—the known VH subclasses, as is the case in the proteins reported here, or very different from the usual amino terminus[3]. This complexity precludes any generalisations as to possible mechanisms responsible for these findings and has been discussed previously[18]. The constancy of the remainder of the Fc fragment as reflected by the relatively infrequent occurrence of additional mutations in CH2 and CH3 domains has also been emphasised before and is further supported by the sequence of protein WIS.

The unique structure of the hinge contrasts with the striking homologies of the four H chain domains. Its basic subunit is 15 residues long, rich in proline and contains the inter-heavy chain disulphide bridges and sometimes the HL bridge[15]. Its origin is unknown; it could be part of a gene coding for a domain which has undergone deletions and mutations, it could be encoded by intervening sequences shown to be present between genes coding for domains[13], or as previously suggested it may be an inserted DNA fragment (transposon) capable of joining duplicated genes which have evolved to perform different biological functions[4], somewhat analogous to the J fragment of the L chain[11]. These features, together with the finding that the hinge often marks the end of the internal deletions[9], that it can be deleted selectively[5,9], and that it is a site where duplications and unequal crossing over occur[16], have given rise to the suggestion that the hinge may be under the control of a separate genetic unit. This possibility has received strong support from the recent observation that non-coding DNA segments occur not only between the domains but also at each side of the hinge in the case of cloned DNA for murine γ-chains[13] (Fig. 2*a*).

Eukaryotic genes, including immunoglobulin L and H chain genes, are organised into transcriptional units in which coding segments of DNA (exons) alternate with non-coding sequences (intervening sequences or introns) to be excised from the primary transcript[10–13,19]. The organisation of the immunoglobulin genes seems to be unique in that the intervening sequences apparently separate exons coding for structural subunits or domains. In the case of the cloned DNA coding for the constant region of the mouse γ1 heavy chain, electron microscopy and sequence analysis revealed four exons separated by four intervening sequences[13] (Fig. 2*a*). Comparison with the known amino acid sequence[20] demonstrated that the exons represent the three CH domains and the hinge, and that no other intervening sequences exist within coding DNA segments. However, because of the complexity of human γ3 hinge gene(s), additional intervening sequences and splicing sites can be anticipated between the subunits of its quadruplicated hinge region (Fig. 2*b*, H region). In addition, based on the unusual V region sequences in γ deletion mutants and the sequence of protein WIS (see Fig. 1, residues 8–11), intervening sequences similar to those described for the mouse λ-chain gene[10,11] (Fig. 2*b*) may also exist near the amino terminus of the V gene and at the J region. Because the intervening sequences are excised from the precursor and the coding regions rejoined, there must be some recognition signal at the junction between the intervening and the coding sequences to permit the accurate excision and splicing necessary to produce the mature mRNA[21]. Such seems to be the case for the repetitive subunits of the hinge genes which, as pointed out before, have kept sequence signals similar to those at the joining sites between VJ and C genes of the L chains[22].

In both H and L chain deletion mutants there is a striking correlation between the borders of deletions and the recently defined points of splicing. Figure 2 correlates the splicing points defined for mouse γ1 constant genes with sites previously described for recombination in deleted H chains. There is also similar striking correspondence for fragmented L chain genes and two L chain mutants[8] which end at the intervening sequences bordering the J region. If sites of splicing determine where deletions can end, as seems to be the case in all mutants studied so far, the finding that in protein ZUC the deletion ends at the last subunit rather than the beginning of the quadruplicated hinge provides additional support for the existence of splicing points and intervening sequences between the subunits of the hinge (Fig. 2*b*).

Although the mechanisms responsible for the deletions are not known, the defect could occur either at the DNA level or during splicing events when heterogeneous nuclear RNA is converted to mature mRNA. A mutation at or near a splicing site could alter the splicing pattern so that the entire coding region can be skipped in the mRNA; alternatively, if the splicing enzyme(s) can bridge non-adjacent encoded regions, large deletions involving more than one genetic unit could result in mutant proteins in which more than a single domain can be eliminated. Studies of immunoglobulin variants, together with DNA cloning of selected mutant cells, will undoubtedly continue to provide clues to the genetic mechanisms involved in H and L chain control and pose interesting questions about the evolution and significance of eukaryotic fragmented genes.

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Suppression of the nonsense mutation in homozygous β^0 thalassaemia

Judy C. Chang, Gary F. Temple, Richard F. Trecartin & Yuet Wai Kan

Howard Hughes Medical Institute Laboratory and Hematology Service,
San Francisco General Hospital, University of California,
San Francisco, California 94143

The common form of β thalassaemia associated with elevated haemoglobin A_2 levels can be broadly classified as β^+ or β^0 type according to the presence or absence of β-globin chain synthesis in the homozygous state[1–4]. The molecular pathology of each type is heterogeneous[3–9]. Apart from a subgroup of Indo-Pakistani patients[10], the β-globin structural gene is intact in the majority of patients with β^0 thalassaemia[2–4]. The amount of β-globin mRNA present in the reticulocytes of these patients varies[5–7]: in some it is absent or barely detectable; in others, a substantial amount is present, but it is nonfunctional. We recently demonstrated that the molecular lesion in a Chinese patient with nonfunctional β-globin mRNA[11,12] was due to the mutation of the normal lysine codon AAG at amino acid 17 to the amber terminator codon UAG, which prematurely terminates the β-globin chain[13]. In the present study we demonstrate the first example of a nonsense mutation in humans which can be suppressed *in vitro* by the suppressor tRNA, as has been found in other eukaryotic cells and viruses[14,15].

The *in vitro* translation of the globin mRNAs prepared from the reticulocytes of the patient with homozygous β^0 thalassaemia was performed in the micrococcal nuclease-treated rabbit reticulocyte lysate system using ^{35}S-methionine as the radioactive amino acid[16]. The globin chains synthesised were separated on a carboxymethyl cellulose column[17] (Fig. 1). The patient's mRNAs directed the synthesis of α- and γ-globin chains but no β-globin chain in the rabbit reticulocyte system. When a serine-inserting amber suppressor tRNA which recognises the codon UAG was added to the mRNA in the cell-free system a new radioactive peak appeared. Its radioactivity was about one-twentieth of that of α-globin. This new polypeptide chain eluted from the column earlier than the β^A-globin marker, consistent with the insertion of serine instead of lysine in amino acid number 17 by the serine suppressor tRNA. We did not detect any additional peaks as the normal termination codons of α-(UAA) (ref. 18) and γ-globin (UGA) (ref. 19) would not be suppressed by this tRNA. As a control, we added this suppressor tRNA to the mRNA from a patient with sickle cell anaemia and detected no new radioactive peak (data not shown).

To confirm that the newly synthesised polypeptide from the β^0 thalassaemia mRNA was a β-globin chain, we digested the polypeptide with trypsin, separated the tryptic peptides by high voltage paper electrophoresis[20], and identified the ^{35}S-methionine-containing peptide, βT5 (amino acid numbers 41–59). The βT5, from the newly synthesised chain, migrated to the same position as that of the β^S chain, confirming that it was indeed a β-globin chain (Fig. 2).

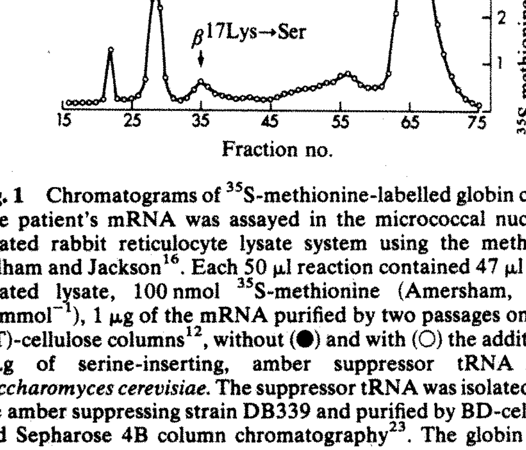

Fig. 1 Chromatograms of ^{35}S-methionine-labelled globin chains. The patient's mRNA was assayed in the micrococcal nuclease-treated rabbit reticulocyte lysate system using the method of Pelham and Jackson[16]. Each 50 µl reaction contained 47 µl of the treated lysate, 100 nmol ^{35}S-methionine (Amersham, 1,000 Ci mmol^{-1}), 1 µg of the mRNA purified by two passages on oligo (dT)-cellulose columns[12], without (●) and with (○) the addition of 6 µg of serine-inserting, amber suppressor tRNA from *Saccharomyces cerevisiae*. The suppressor tRNA was isolated from the amber suppressing strain DB339 and purified by BD-cellulose and Sepharose 4B column chromatography[23]. The globin chain products were separated on a carboxymethylcellulose column as previously described[17]. The position of elution of normal γ-, β^A- and α-globin chains was determined by optical density measurements of 5 mg of HbA and F added as carrier.

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The amount of β-globin mRNA present in this patient's reticulocytes was 15% of the α-globin mRNA[4]. Since the efficiency of the suppressor is generally not more than 60% (refs 21, 22), the amount of β-relative to α-globin synthesis would be about 10%. The finding of a $\beta:\alpha$ ^{35}S-methionine radioactivity ratio of 1:20 is consistent with this since β-globin chain contains one, and the α-globin two methionine residues. The low level of β-globin mRNA may be due to premature degradation of the mRNA, as a result of lack of ribosomes over nearly 90% of the coding region following the nonsense mutation. Such a mRNA would be more susceptible than normal to nuclease degradation within the erythroid cell. In addition, the decreased β-globin mRNA could also be partially explained if one of this patient's β-globin alleles contained the nonsense mutation while the other contained a different kind of mutation which results in the absence of mature β-globin mRNA from that gene.

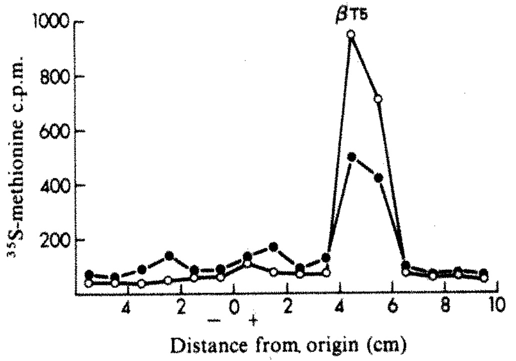

Fig. 2 High voltage electrophoresis of ^{35}S-methionine-labelled tryptic peptides. The β-globin peaks from cell-free synthesis products with mRNA from the β^0 thalassemic patient (●) and a patient with sickle cell anaemia (○) were desalted through a Sephadex G-25 column in 0.5% formic acid, and lyophilised. 200 μg of globin from each peak was incubated in 0.5 ml of 1% NH_4HCO_3 at 37° C with 8 μg of trypsin (Worthington TPCK-treated, 259 units mg^{-1} for 4 h. The hydrolysate was twice lyophilised and dissolved in 10 μl of pyridine acetate buffer (pyridine: acetic acid: water 25:0.3:75 by volume), *p*H 6.58. The peptides were separated by electrophoresis on 3 MM paper at 2,000 V for 2 h, 1-cm strips of the paper were cut out and the radioactivity measured in a Beckman liquid scintillation counter.

This study corroborates our sequence analysis data on this patient which indicated that β^0 thalassaemia is the result of a nonsense mutation within the β-globin structural gene[13]. To date, only one likely example of a nonsense mutation in higher eukaryotic cells has been reported. Capecchi *et al.* described a mouse cell line deficient in the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT)[14]. The mutant enzyme was probably shortened at the carboxy terminus. Fusion of the defective cells with erythrocytes preloaded with either *Escherichia coli* or yeast suppressor tRNAs restored HGPRT activity when the erythrocytes contained ochre (UAA) but not amber (UAG) suppressor tRNA. Our results indicate that a defined nonsense mutation in human β-globin mRNA can be suppressed *in vitro* using the appropriate yeast suppressor tRNA. Similarly, delivery of suppressor tRNA to the erythroid cell precursor of patients with β^0 thalassaemia due to nonsense mutations may correct the β-globin defect. This approach may offer new methods for treatment of this disorder.

It is likely that nonsense mutations may have occurred at different locations along the β-globin mRNA in other β^0 thalassaemias. The use of suppressor tRNAs will facilitate the detection of nonsense mutations in the various thalassaemia syndromes as well as in other genetic disorders where a specific protein is deficient or absent.

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Heterozygosity of *H–2* loci in wild mice

William R. Duncan

Department of Cell Biology, The University of Texas Health Science Center, 5323 Harry Hines Blvd, Dallas, Texas 75235

Edward K. Wakeland & Jan Klein

Abteilung Immungenetik, Max-Planck-Institut für Biologie, Corrensstr. 42, 7400 Tübingen, FRG

The major histocompatibility complex (MHC) is a cluster of genetic loci coding for cell surface molecules that seem to be the second most important element of the vertebrate immune system after immunoglobulins[1]. The MHC loci can be divided into three classes. Class I loci code for molecules of about 44,000 molecular weight (MW) which seem to function as markers of self in T-lymphocyte-mediated cytotoxicity. Class II loci code for molecules consisting of two polypeptide chains, of MW 34,000 and 28,000 respectively, which are believed to regulate the immune response by controlling the behaviour of helper and suppressor T lymphocytes. Each molecule may carry several antigens—one 'private' (characteristic for the allele controlling this molecule) and several 'public' ones[2] (shared with molecules controlled by other alleles). Class III loci code for serum proteins. One of the most characteristic and remarkable properties of the MHC loci, in particular class I, is their genetic polymorphism, defined as the existence in a population of two or more alleles at appreciable frequencies[3–5]. In *H-2*, the MHC of the mouse, individual alleles at class I *K* and *D* loci occur in the two populations that have been studied thus far with an average frequency of about 2% (ref. 5). We estimate, therefore, that these two populations contain some 100 alleles at each of the two loci. The polymorphism of class II locus *A* seems to be somewhat lower than that of the class I loci (average allelic frequency of 2–5%), while at the second class II locus (*E*) only five alleles have been identified[5]. The polymorphism implies that a high degree of heterozygosity should exist at the MHC loci in natural populations, and this has been found at the *HLA* loci, the human MHC (for review see ref. 6). We report here a similarly high frequency of heterozygosity in the mouse *H-2* complex in a population of mice, in spite of a social structure that would tend to reduce heterozygosity.

Table 1 Class I H-2 antigens of wild mice and their progeny

Wild mouse	No. of progeny	Phenotype	Wild mouse	No. of progeny	Phenotype
CC27	—	105, 115, 117	HGE61	—	2
	2	105, 115, 117		1	2
	3	—		3	—
DF55	—	17, 103, 118	AU136	—	19
	1	17		2	19
	1	103, 118		4	—
WD104	—	9, 103, 118	KF72	—	2
	2	103, 118		1	2
	1	9		1	—
DFW36	—	9	DF85	—	20, 105, 114
	3	9		2	20
	2	—			
DFW33	—	9, 20, 103, 116, 118	OS9	—	16, 115, 117
	2	9, 20, 103, 118		1	16
	2	116		2	115, 117
PD121	—	119, 120	CC16	—	118, 119, 120
	2	119, 120		1	118, 119, 120
	3	—		3	—
DF48	—	119, 120	MD3	—	108, 111, 119, 120
	1	119, 120		3	108, 111, 119, 120
	3	—		1	—
DF89	—	116	DF45	—	25, 118
	2	116		1	118
	2	—		1	25, 118
CC21	—	115, 117	DF92	—	2, 16, 117, 119, 120
	2	115, 117		3	119, 120
	2	—		2	2, 16, 117
MS8	—	105, 115, 117	BCD131	—	119, 120
	1	105		1	119, 120
	1	115, 117		3	—
MD7	—	19	DWD130	—	103, 118
	1	19		3	103, 118
	1	—		1	—
OS40	—	16, 31, 105, 115, 117	DF88	—	20, 25, 109
	3	16		6	20, 25, 109
	1	31, 105, 115, 117			

Wild males were mated with MWT ($H\text{-}2^b$) females and tested, together with their progeny, in the cytotoxic test against a panel of operationally monospecific antisera. Positive reactions for private and semiprivate antigens listed under Phenotype were found.

There are two ways of estimating *H-2* heterozygosity. First, if all private H-2 antigens and the loci coding for them in a given wild mouse sample were known, one could determine the heterozygosity of each locus simply by counting individuals carrying two allelically determined private antigens. Because, however, we do not know all the private H-2 antigens and we often do not know the identity of loci (*H-2K* versus *H-2D*) controlling them, we can only use this method to estimate the minimal frequency of *H-2* heterozygotes. As there are two major class I and two major class II loci, the presence of three private class I or class II antigens in an individual must mean that this individual is heterozygous in at least one class I (or class II) locus. Similarly, simultaneous presence of four private class I or class II antigens in an individual can be interpreted to mean that this individual is heterozygous at the two class I or class II loci. Individuals with two or less private antigens may or may not be heterozygotes, and it is this uncertainty that makes the estimate minimal.

Wild mice were captured at 15 different localities in Texas, and typed in the dye-exclusion cytotoxic test with 49 H-2 antisera and 12 Ia sera, which detected 36 private class I and 10 class II antigens[5]. In this sample of 88 mice, 31 mice carried more than two private class I antigens (data not shown). Thus, the minimal frequency of *H-2* heterozygotes at class I loci among Texas mice is 35%.

The second way of estimating *H-2* heterozygosity involves crossing wild males (wild female mice do not usually mate in captivity) with an *H-2* homozygous testing stock of laboratory mice, and *H-2*-typing the progeny of this cross. Segregation of private H-2 antigens among the progeny indicates heterozygosity of the wild father. This method fails only in cases where the father does not carry any known private antigens or where one of the paternal *H-2* chromosomes carries a strong segregation distorter, such as that present in the *H-2* linked *t* complex[7].

This second approach was applied to 26 of the mice captured in Texas, which were mated with $H\text{-}2^b$ homozygous MWT stock. From these matings 90 hybrids and their fathers were obtained and typed with the above panel of H-2 and Ia antisera (Table 1). Two of the 26 wild males (and their progeny) did not carry any of the private class I antigens detected with our battery of antisera. Of the remaining 24 males, private H-2 antigens segregated in the progeny of 23; the offspring (six) of only one mouse (DF 88) all carried the same class I antigens as their father. Provided that this result was not caused by segregation distortion, this one mouse might have been an *H-2* homozygote. Thus, the overall frequency of class I heterozygotes in the sample was 96%. In seven of the wild fathers private antigens were present that are known to be controlled by the *H-2K* locus. All seven mice proved to be heterozygous at this locus. Similarly, in four mice, antigens known to be controlled by the *H-2D* locus were found, and these mice were also all *H-2D* heterozygotes. With regard to class II antigens, it was possible to type 10 mice for Ia-1 and 19 mice for Ia-5 antigens. Eight of the Ia-1 and nine of the Ia-5 mice were heterozygous. Hence, the frequency of heterozygotes at *Ia-1* and *Ia-5* loci is 80 and 47%, respectively (Table 2).

This high degree of heterozygosity is unprecedented in a wild mouse population. The average frequency of heterozygotes at all other polymorphic loci tested is about 6%, with the highest recorded frequency of 28.6% for malic enzyme[8]. The *H-2* far exceeds this frequency. In fact, in the *H-2* system, maximum heterozygosity is achieved in that, at least for the class I loci, virtually all the mice are heterozygous.

Table 2 Number of wild mice heterozygous at individual *H-2* loci

Locus	Heterozygous total tested	% Heterozygotes
H-2K	7/7	100
H-2D	4/4	100
Class I*	23/24	96
Ia-1	8/10	80
Ia-5	9/19	47
All *H-2*†	25/26	96

*Includes mice in which *H-2K* and *H-2D* loci could not be distinguished phenotypically.

† If one of the four loci tested was heterozygous, the mouse was counted as a heterozygote.

How does this high *H-2* heterozygosity of natural mouse populations arise? Were the mouse population of the random mating type, such a high frequency of *H-2* heterozyotes would be expected. If the frequency of individual *H-2* alleles is—as preliminary data[5] suggest—about 2%, and if there are approximately 100 alleles at each of the three highly polymorphic *H-2* loci (*H-2K*, *H-2D* and *Ia-1*), then the expected frequency of *H-2* heterozygotes would be nearly 100%. However, it is known that mouse populations are not of the random mating type. On the contrary, they are broken down into family units or demes, each family consisting of one dominant male, a few subordinate males and a few females (for review see ref. 9). The demes are more or less closed, with little evidence for interdemic migration. Although there is almost no evidence indicating that mice in Texas live in deme-type populations, there is no reason to believe that they are different in this respect from mice in other geographical areas. Furthermore, preliminary data on mice from Germany indicate that these too display a high degree of *H-2* heterozygosity (unpublished). Thus, the phenomenon seems to be of general validity.

The deme structure of the mouse population and the high degree of heterozygosity need to be reconciled. There is considerable evidence indicating that mice within a deme (or locality) genetically resemble one another more than mice of different demes (see ref. 9). This observation, which also applies to minor histocompatibility loci[10] (that is, *H* loci that are not members of the MHC), suggests that a certain degree of inbreeding occurs within a deme. The intrademic inbreeding should lower the heterozygosity at the *H-2* loci, the degree of the decrease depending on the inbreeding coefficient. As the latter is not known for the population tested, we cannot estimate how large a decrease to expect. Should the value of the inbreeding coefficient in this population prove to be far from negligible, the fact that near 100% *H-2* heterozygosity occurs would mean that the effect of inbreeding is probably offset by heterozygous advantage at the *H-2* loci.

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Evolutionary nucleotide replacements in DNA

Thomas H. Jukes & Jack Lester King

Department of Biophysics and Medical Physics,
University of California, Berkeley, California 94720
Department of Biology,
University of California, Santa Barbara, California 93106

With the increasing availability of analytical information on mRNA molecules, it is now possible to compare homologous nucleotide sequences from different organisms and to draw conclusions about their evolution. Such comparisons[1,2] have shown that silent changes in codons occur more frequently than nucleotide replacements that produce changes in amino acid sequences (code-altering changes). Furthermore, there is an important difference between amino acid sequence comparisons and nucleotide sequence comparisons. The former show only differences in amino acid residues, but the latter show several types of differences when corresponding codons are compared[3]. Single-base replacements may be degenerate (silent) or expressed as amino acid replacements. Two-base codon changes may be degenerate, single-base changes, or be visible as such. Three-base codon changes may be degenerate (involving serine), simulate either single-base or two-base changes or be visible as such. All nine types of change are found in comparisons of genes from the viruses ΦX174 and G4. The relative numbers of these nine types as based on all possible interchanges between all 61 amino acid codons were listed by Holmquist *et al.*[3] and are shown in Table 1. We discuss these results in the light of the significance of nucleotide changes in molecular evolution.

Theoretically, there are 526 possible single-base changes (substitutions) in the 61 codons for the 20 amino acids; 392 produce amino acid changes ('code-altering replacements') and 134 of the changes are silent. Changes that produce chain-terminating codons are omitted from this calculation, because such changes will be rejected. As a result, in a comparison of two homologous genes, one would expect to find on a random basis 25% of silent evolutionary single-base replacements. The calculations for codon changes involving two or three nucleotide replacements are complicated by the fact that such changes will often include more than one change at the same nucleotide site, some of which will be revertants[3]. A value much higher than 25% of silent replacements among single-base changes would show that code-altering changes are adopted more slowly than silent changes because of negative selection. The reverse finding would show evolutionary pressure against the adoption of silent changes as replacements. Table 1 shows that in three of the four summarised examples, code-altering single-base changes are 1.8–3.0 times as likely to be rejected as are silent changes. It could be suggested that silent changes are adopted rapidly because they are advantageous rather than neutral. However, advantageous changes can be incorporated only slowly during evolution because of substitutional load[4], so this reason for the rapid adoption of silent changes is unlikely.

The fourth example consists of genes *B*, *E* and *K* of ΦX174 and G4. These are the three genes that are read as frameshifts in genes *A*, *C* and *D*. Barrell *et al.*[5] and Godson *et al.*[6] concluded that the gene *E* arose at a later stage of evolution than gene *D*. This would be consistent with the much lower percentage of silent changes in gene *E* (ref. 6). Such changes in gene *E* would nearly all produce amino acid changes in the predecessor gene *D*, because third positions of codons in gene *E* are first positions of codons in gene *D*. A similar reasoning can be applied to gene *K* as compared with its predecessors *A* and *C*, and to gene *B*, in which third codon positions are second positions of codons in gene *A* (refs. 5, 6). Therefore, silent changes in genes *B*, *K* and *E* would often be rejected as causing unacceptable amino acid changes in genes *A*, *C* and *D*. Indeed, a markedly low value for the per cent of silent changes might indicate *per se* that a gene is being read by a frameshift in a gene of earlier origin. The results in Table 1 show that with the predictable exceptions of genes *B*, *K* and *E*, the genes in phages ΦX174 and G4 have incorporated silent nucleotide replacements much more rapidly than code-altering replacements.

The question arises, are silent replacements actually neutral, or have they taken place for adaptive reasons, such as a requirement for a specific secondary stucture in mRNA, or a preferential use for certain transfer RNAs in regulating the rate of synthesis of a protein? Modulation of the rate of translation by isoacceptor tRNAs, and adaptation of tRNA pattern to the demands of translation were among the reasons advanced by Kafatos *et al.*[7] to explain the nonrandom use of synonymous codons in rabbit and human β-globin mRNAs. However, such arguments are unconvincing, for each β-globin mRNA has a different list of usage for synonymous codons. It seems highly improbable that closely related mammals would have different requirements for mRNA structure and tRNA usage for the same protein. If this were so, a staggering number of species differences, each necessitating a separate evolutionary pathway, would be needed at the molecular level for the thousands of different homologous proteins in each species. We therefore conclude that silent single-base replacements in the codons of β-globin mRNAs are probably neutral. This simple explanation probably also applies to the other comparisons of mRNAs in Table 1.

We have previously stated[8] that 'if DNA divergence in evolution includes the random fixation of neutral mutations, then the third position nucleotides should change more rapidly, because synonymous mutations are more likely to be neutral'. This prediction agrees with the findings summarised in Table 1, with the exception, for reasons noted, of the 'frameshift genes'. Recently, Kimura[1] has drawn attention to the preponderance of silent replacements over code-altering replacements as evidence for the neutral theory of molecular evolution, based on comparisons of 53 nucleotide positions in human and rabbit β-globin mRNAs plus 84 positions in histone H4 mRNAs of two sea-urchin species. We have extended and expanded Kimura's findings, and Table 1 shows comparisons of 8,469 nucleotide positions.

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Table 1 Evolutionary replacements in mRNAs and viral genes compared with interchanges in code

		β-Globins	α- and β-Globins	ΦX174 vs G4 genes		Possible interchanges in code table
				In phase	Frameshift	
Single-base	Example					
Degenerate	UUU/UUC	84	64	487	16	134
Recognisable	UUU/AUU	27	78	160	77	392
Per cent silent		76	45	75	17	25
Two-base						
Degenerate	CUC/UUA	2		31		28
Recorded as single-base	UUU/AUC	30	95	151	17	1,006
Recognisable	UUU/GAU	13	25	80	14	534
Three-base						
Degenerate	UCU/AGC			4		12
Recorded as single-base	UCU/GGC	1	2	29		308
Recorded as two-base	UCU/GUA	2	44	124	8	1,164
Recognisable	UUU/AAG		1	6		82
Totals						
Nucleotides compared		1,323	1,251	5,094	801	10,980
Nucleotide replacements		210	523	1,660	178	8,360
Silent replacements		122	207	894	41	3,012
Per cent silent replacements		58	40	54	23	36

The globin comparisons are of mRNA sequences from refs 7, 9, 10, 11 and 12. The β-globins are of rabbit, human and mouse and were compared pairwise. The α-globin was of rabbit, and was compared with the three β-globins. The gene sequences of ΦX174 and G4 are from refs. 5 and 6. The comparisons of the codon table are from ref. 3, and are the total of possible amino acid codon changes in both directions, excluding changes that involve chain-terminating codons.

It is obvious from results now available that the inaccurate and crude procedure of measuring evolutionary changes in terms of 'minimum base differences per codon' is defective and therefore obsolete.

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Note added in proof: Friedman *et al.*[13] compared the sequences of the early regions of polyoma and SV40 genomes. Our examination of their Fig. 4 shows 65% silent single-base changes, and 43% silent replacements in 2,079 nucleotide comparisons with 1,080 replacements.

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Localisation of the $^{G}\gamma$-, $^{A}\gamma$-, δ- and β-globin genes on the short arm of human chromosome 11

Alec J. Jeffreys*†, Ian W. Craig‡ & Uta Francke§

† Genetics Department, University of Leicester, University Road, Leicester, UK
‡ Genetics Laboratory, Department of Biochemistry, University of Oxford, South Parks Road, Oxford, UK
§ Department of Human Genetics, Yale University School of Medicine, 333, Cedar Street, New Haven, Connecticut 06510

Human–mouse somatic cell hybrids have proved invaluable in assigning human genes to their respective human chromosomes[1]. To date, the success of this approach has depended on identifying human proteins which are synthesised in hybrid cells containing a small number of human chromosomes. Consequently, chromosome assignment has been limited mainly to human proteins which are expressed in man–mouse somatic cell hybrids and for which a suitable assay, usually electrophoretic or immunological, exists to distinguish between the human and murine homologous proteins. This technique is therefore unsuitable for the assignment of those human genes which are expressed only in differentiated cells and not in hybrid cells. Here, we describe how nucleic acid hybridisation and restriction endonuclease mapping of DNA can be combined to test for the presence of human structural gene sequences within hybrid cell DNA. This method can be used to assign any purified human DNA sequence to a human chromosome, and does not require the DNA sequence to be expressed in man–mouse hybrid cells.

The genes that we have studied are those which code for the $^{G}\gamma$- and $^{A}\gamma$-globin polypeptides of human fetal haemoglobin (HbF, $\alpha_2{}^{G}\gamma_2$ and $\alpha_2{}^{A}\gamma_2$) and those which code for the β- and δ-globins of major and minor adult haemoglobin (HbA, $\alpha_2\beta_2$ and HbA$_2$, $\alpha_2\delta_2$, respectively). DNA fragments containing the β- and δ-globin genes can be detected in restriction endonuclease digests of total human DNA by agarose gel electrophoresis of the digested DNA, transfer of the digest products to a nitrocellulose filter by blotting[2] and hybridisation of the filter with ^{32}P-labelled cloned complementary DNA (cDNA) made from human β-globin messenger RNA (plasmid pHβG1 (ref. 3)). The globin DNA fragments detected have been ordered into a physical map of restriction endonuclease cleavage sites around the human β- and δ-globin genes[4]. Similarly, cloned

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* To whom all enquiries should be addressed.

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cDNA made from human $^{G}\gamma$-globin mRNA (plasmid pHγG1 (ref. 3)) has been used as a nucleic acid hybridisation probe to construct a physical map around the $^{G}\gamma$- and $^{A}\gamma$-globin genes[5].

To assign these genes to a human chromosome, we therefore prepared DNA from a panel of human–mouse somatic cell hybrids, and digested the DNA with a restriction endonuclease which would release the β-related globin genes in DNA fragments of a convenient size for subsequent detection by electrophoresis and filter hybridisation with either ^{32}P-labelled pHβG1 DNA or pHγG1 DNA. The restriction endonucleases chosen were *Pst*I, which liberates the β- and δ-globin genes in DNA fragments of 5.0 and 2.3 kilobase pairs, respectively[4], and *Hind*III, which generates a 8.2-kilobase $^{G}\gamma$-globin DNA fragment and a 3.5-kilobase $^{A}\gamma$-globin fragment[6].

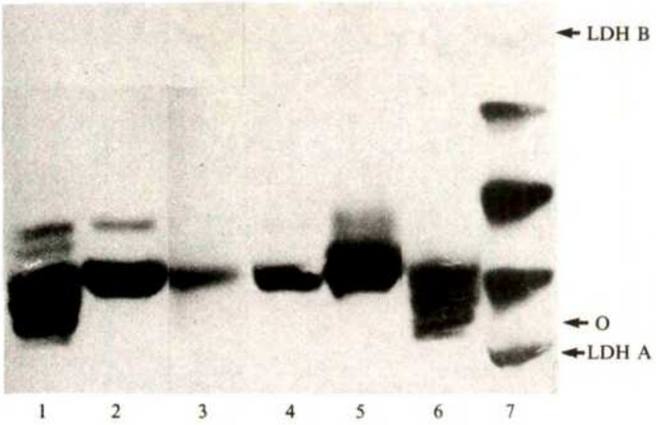

Fig. 1 Electrophoretic analysis of LDH in man–mouse somatic cell hybrids used in this study. Hybrid cell extracts were electrophoresed on cellulose acetate strips and stained for LDH activity as described elsewhere[12]. Cells used were: (1) LBT.2; (2) mouse (3T3 cells); (3) LBT.2 grown in the presence of BUdR; (4) mouse (1R cells); (5) Cl.5; (6) LA.4; (7) human (HeLa cells). The electrophoresis origin (O) and positions of human LDH A and LDH B homotetramers are as indicated. Hybrids LA.4 and LBT.2 exhibit a multiple banded pattern characteristic of cells expressing human LDH A in addition to mouse LDH.

Early in this project we learned that Deisseroth *et al.* had used solution hybridisation of labelled human β- and γ-globin cDNA with man–mouse hybrid cell DNA to show that β- and γ-globin genes were located on human chromosome 11 (ref. 7). We therefore limited our investigation to DNA prepared from the four somatic cell hybrid clones described in Table 1. The hybrid LA.4 has been shown by karyotyping to contain human chromosomes 11 and X (ref. 8); as shown in Fig. 1, this hybrid cell line also expresses human lactate dehydrogenase A (LDH A) determined by the short arm of human chromosome 11 (ref. 9). A subclone of LA.4, termed Cl.5, had lost human chromosome 11 and therefore the ability to synthesise LDH A. A third hybrid clone, LBT.2, contained a human translocation chromosome comprised of the short arm of chromosome 11 and most of the long arm of chromosome 17 (11 cen → 11p15; 17q21 → 17 qter)[9]. This chromosome enables LBT.2 cells to synthesise human LDH A and also codes for the soluble form of thymidine kinase (TK); a gene necessary for the expression of this enzyme has been assigned to the long arm of human chromosome 17 (ref. 10). The fourth cell line was derived by growing LBT.2 in bromodeoxyuridine (BUdR) to select against cells carrying the TK gene; this cell line, termed LBT.2-BUdR, had, as predicted, lost the human translocation chromosome and the ability to synthesise human LDH A.

DNA was prepared from each of these cell lines, digested with restriction endonucleases *Pst*I or *Hind*III and compared with restricted human and mouse DNA by agarose gel electrophoresis, transfer to a nitrocellulose filter and subsequent hybridisation with ^{32}P-labelled pHβG1 or pHγG1 DNA (Fig. 2). In the conditions of hybridisation used, each probe can readily detect its corresponding globin DNA fragments in 10 μg digested human DNA. These human probes show little or no hybridisation to mouse DNA. In a reconstitution experiment in which human and mouse DNA were mixed in varying proportions before restriction and hybridisation, it can be seen that all β-related globin genes could be detected in human–mouse DNA mixtures containing as little as 10% of the normal level of human globin genes. Most important, $^{G}\gamma$-, $^{A}\gamma$-, δ- and β-globin DNA fragments were clearly detectable in hybrid cell lines LA.4 and LBT.2, which contain human chromosome 11 and the short arm of 11, respectively, and were absent in DNA prepared from Cl.5 and LBT.2-BUdR cell lines, neither of which contain chromosome 11. This experiment confirms the assignment by Deisseroth *et al.* of the human γ- and β-globin genes to human chromosome 11 (ref. 7). It also demonstrates that all four β-related globin genes are located within the short arm of chromosome 11 within the region 11 cen → 11p15 (ref. 9). This assignment is formally established by the segregation of the globin genes during selection against the linked TK marker in LBT. 2 cells grown in the presence of BUdR.

Table 1 Man–mouse somatic cell hybrids used in the present study

Hybrid cell line	Human chromosomes identified	Human LDH A	β-Related human globin DNA fragments
LA.4	11, X	+	+
Cl.5	X	–	–
LBT.2	t(11 : 17)(p15; q21)	+	+
LBT.2-BUdR	—	–	–

Hybrid cell lines LA.4, Cl.5 and LBT.2 were grown in RPMI 1640 medium supplemented with 100 μM hypoxanthine, 10 μM methotrexate and 16 μM thymidine. LBT.2–BUdR was obtained by culturing LTB.2 cells for 12 transfers in RPMI 1640 medium supplemented with 30 μg ml^{-1} BUdR. The human chromosome constitution of the cell lines was confirmed by Giemsa 11 and trypsin banding analysis of metaphase preparations (M. Bobrow, personal communication). >90% of LBT.2 cells contained the human translocation chromosome. Detailed quantitative estimates of proportions of LA.4 and Cl.5 cells which contain the indicated human chromosomes are not available. The presence or absence of human LDH A was assayed by electrophoresis of hybrid cell extracts on cellulose acetate (Fig. 1). The analysis of β-related human globin DNA fragments is described in Fig. 2 legend.

This approach to assigning human genes to human chromosomes is simple, fast and only requires that a suitable purified DNA be available as a nucleic acid hybridisation probe. It is also sensitive and enough DNA can be isolated from 10^7 man–mouse hybrid cells to test for the absence or presence of structural gene sequences. In this study the assignment was aided by a very low level of cross-hybridisation between the human globin-gene probes and mouse DNA in the hybridisation conditions used. However, this approach could also be extended to probes which hybridise equally well to both human and mouse DNA, and only requires a restriction endonuclease which generates different electrophoretic patterns of hybridisable DNA fragments from human and mouse DNA. With the advent of libraries of cloned human DNA fragments[11], this assignment procedure could be used to identify cloned DNA fragments from any required human chromosome.

The assignment of human genes to chromosomes by analysing DNA sequences has two major advantages over the alternative approach of studying the production of human proteins in man–mouse somatic cell hybrids. First, it does not require expression of human genes and is therefore suitable for the assignment of genes coding for proteins such as globins which

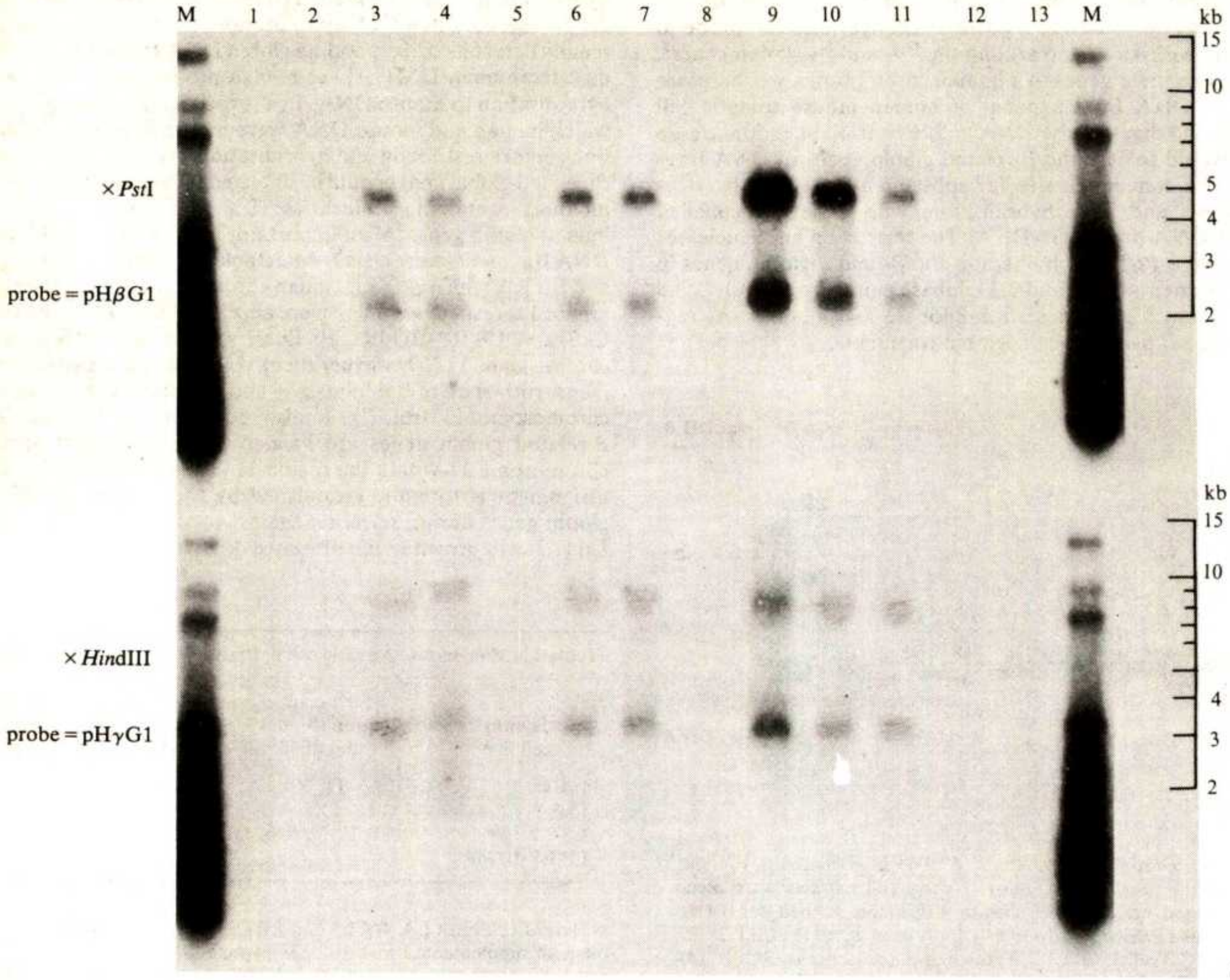

Fig. 2 Analysis of human β-related globin DNA fragments present in restriction endonuclease digests of man–mouse somatic cell hybrid DNA. DNA was prepared from human placenta, from BALB/c mouse brain, from cultured 1R cells and from four man–mouse somatic cell hybrids, using methods described elsewhere[13]. Batches (10 μg) of DNA were digested with endonuclease *Pst*I or *Hin*dIII (Microbiological Research Establishment, Porton Down). DNA digests were recovered, alkali-denatured, electrophoresed through a 1% agarose slab gel and transferred by blotting to a nitrocellulose filter. pHβG1 and pHγG1 DNA were labelled with ^{32}P *in vitro* by nick translation with [α-^{32}P]dCTP (2,000–3,000 Ci mmol^{-1}, Amersham) to a specific activity of 1.5×10^8 d.p.m. per μg DNA. After pretreatment of the filters to prevent nonspecific binding of single-stranded DNA, the filters were hybridised for 2 d at 65 °C in 1 × SSC (saline sodium citrate; 0.15 M NaCl, 15 mM trisodium citrate (*p*H 7.0)) with 30 ng ml^{-1} heat-denatured labelled DNA. Unbound labelled DNA was then removed by washing at 65 °C in 1 × SSC and nonspecific DNA hybrids removed by a stringent wash in 0.1 × SSC at 65 °C. Labelled DNA bands were detected by exposing the filters to X-ray film for 15 d in the presence of an intensifying screen. Full details of the preparation of DNA, restriction endonuclease digestion, gel electrophoresis, DNA transfer, nick translation, filter hybridisation, washing and autoradiography are described elsewhere[13]. Samples electrophoresed were: (1) BALB/c DNA; (2) 1R DNA; (3), (4) separate preparations of LA.4 DNA; (5) Cl.5 DNA; (6), (7) separate preparations of LBT.2 DNA; (8) LTB.2-BUdR DNA; (9) human DNA; (10) 1 : 1 human : BALB/c DNA; (11) 1 : 3 human : BALB/c DNA; (12) 1 : 9 human : BALB/c DNA; (13) BALB/c DNA. The hybridisation markers (M) consisted of 10 pg pCRI DNA × *Eco*RI (13.3-kilobase fragments), 10 pg pCRI DNA × *Kpn*I × *Hin*dII (7.3, 1.3, 0.93, 0.86, 0.64, 0.18, <0.1-kilobase fragments) plus 10 pg pCRI DNA × *Eco*Ri × *Hin*dIII (9.8, 3.4-kilobase fragments); pCRI is the plasmid vector used in constructing pHβG1 and pHγG1 (ref. 3).

are only produced by differentiated cells. Second, in hybrid cells in which both human and mouse homologous proteins are being synthesised, assignment depends on distinguishing between the two species' proteins. If the species difference is solely due to differences in the primary amino acid sequence of the proteins, then assignment will lead to the chromosome localisation of the protein's structural gene. On the other hand, if the difference reflects a species-specific difference in, for example, post-synthetic modification of otherwise identical human and mouse proteins, then analysis of man–mouse somatic cell hybrids could result in the assignment, not of the structural genes for the protein, but of the human modification system instead. Direct analysis of DNA sequences and structural genes in the nuclear DNA of somatic cell hybrids avoids this ambiguity and leads directly to the assignment of the structural gene.

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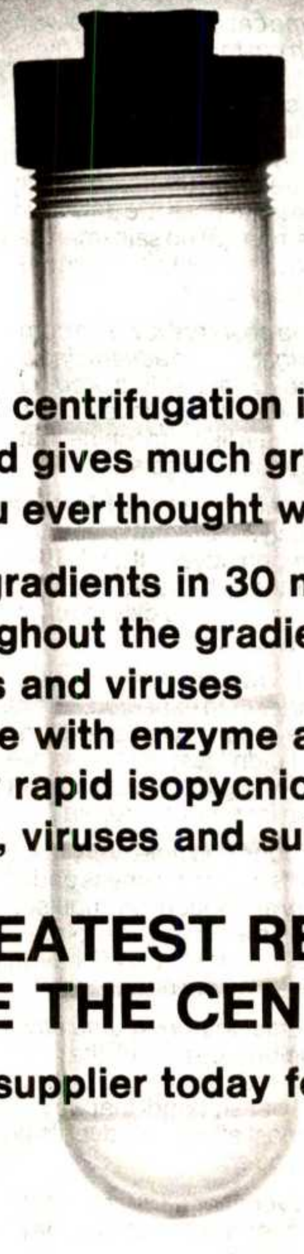

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matters arising

Sm–Nd systematics of Lewisian gneisses and the origin of granulites

THE demonstration by Janardhan *et al.*[1] of the purging of H_2O from previously hydrous hornblende–biotite gneisses by CO_2-rich volatiles of possible mantle origin to form pyroxene gneisses is an important development in the study of deep crustal materials. A popular model to explain the chemical peculiarities of granulite facies gneisses (depletion in K, Rb, Th and U; high K/Rb ratios and low Rb/Sr ratios) has been degassing of their precursors during high grade metamorphism[2–5]. The probability of this having been initiated by increase in p_{CO_2} has been recognised by Tarney and Windley[5]. The degassing model received significant support from Nd isotope studies of the Lewisian gneisses from Scotland[6]. There, Sm–Nd dating revealed an age (2,920±50 Myr) of primary separation from the mantle some 200 Myr older than that of chemical differentiation (loss of Rb and U in particular) recorded by whole rock Rb–Sr and Pb–Pb and zircon U–Pb methods. Hamilton *et al.*[6] imply that the repository for Rb expelled from the granulite facies Lewisian is, in part, the tectonically higher amphibolite facies component of that complex, which now has an Rb/Sr ratio that is far higher than is compatible with their $^{87}Sr/^{86}Sr$ ratio at 2,670 Myr ago[7].

There are important geochemical differences between the granulite and amphibolite facies components of the Lewisian complex that are not discussed by Hamilton *et al.*[6] and to which the discovery of Janardhan *et al.* is relevant. The pyroxene gneisses in the Lewisian of the isles of Tiree and Barra have positive Eu anomalies and abnormally high Sr (average, 837 p.p.m.) whereas tectonically higher hornblende–biotite gneisses have negative Eu anomalies and much less Sr (average, 381 p.p.m.) (refs 4, 9 and unpublished data). Both may be features of a primary igneous fractionation of the gneiss precursors, the pyroxene gneisses representing crystallisation under low p_{H_2O} of a precursor tonalitic magma, the hornblende–biotite gneisses the expelled, residual, hydrous magma[8,9]. The complementary patterns could not reflect simple loss of a hydrous vapour phase from the granulites, but could reflect the addition of Sr and Eu to more normal precursors of the pyroxene gneisses from a mantle-derived vapour phase[4], such as that indicated by Janardhan *et al.*[1].

The dilemma is first that a model involving primary igneous fractionation at low p_{H_2O} (implying high p_{CO_2}) of the pyroxene gneisses can as equally account for the K, Rb, Th and U depleted nature of the Lewisian pyroxene gneisses as for their high Sr and positive Eu anomalies[9], thereby making degassing unnecessary as a means of chemical fractionation. Second, that addition of Sr, Eu and presumably other rare earth elements (REEs) from mantle depths in a CO_2 rich vapour phase would have a significant effect on Sr and Nd isotope systems in pyroxene gneisses.

S. A. DRURY

Department of Earth Sciences,
The Open University,
Milton Keynes, UK

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HAMILTON *ET AL.* REPLY—There may be differences in Sr content between granulite and amphibolite facies gneisses in the Scottish Hebridean Islands, but in the much more extensive outcrop on the mainland, Tarney (ref. 1, Table 1), using a much larger sample population, found only a small difference (615 as against 580 p.p.m. Sr) in gneisses of equivalent major element composition. Moreover, studies of REE distributions in the granulites[2,3] show that it is the more fractionated siliceous gneisses which have the more prominent positive Eu anomalies. The rare earth relationships are very similar to those described recently by Arth *et al.*[4] in a Proterozoic gabbro–diorite–tonalite–trondhjemite plutonic suite from South-West Finland, interpreted as resulting from 'wet' hornblende fractionation. Thus they indicate the opposite petrogenetic relationship from that suggested by Drury[5], but are entirely consistent with the isotope systematics which indicate that the dry granulite facies metamorphism reached its peak some 100–200 Myr after the gneisses were formed. This is also consistent with the conclusions of Janardhan *et al.*[6].

With regard to Drury's dilemma, we can only point out that both granulite facies and amphibolite facies samples define the 2,920±50 Myr Nd isochron. Had there been extensive addition of Sm, Nd and other REEs to the granulite facies gneisses, it seems to us most unlikely that the observed Sm/Nd–$^{143}Nd/^{144}Nd$ corelation would have been maintained. Thus, until we see firm evidence to the contrary, we consider that the normally stable REEs have remained relatively immobile during granulite facies metamorphism, while the commonly mobile elements K, Rb, Th and U have indeed been partially removed.

P. J. HAMILTON
N. M. EVENSEN
R. K. O'NIONS

Lamont-Doherty Geological Observatory
of Columbia University,
Palisades, New York, 10964

J. TARNEY

Department of Geological Sciences,
The University of Birmingham,
Birmingham, UK

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A model for the preferential degassing of the upper mantle

CRITICAL to the model of the depletion of the mantle presented by Hart *et al.*[1] is the assumption that 3.2×10^{-6} ml g^{-1} is an upper limit to the concentration of ^{40}Ar in the mantle source of submarine basalts. This value was obtained from the maximum slope of the upper envelope of data on a plot of ^{40}Ar/^{36}Ar against 1/^{36}Ar. This interpretation ignores the probable significant depletion of initial magma Ar by strong partitioning into CO_2 bubbles and their loss before and during emplacement of magma[2–4]. Further, it has been demonstrated that natural cracks as well as those produced during sample preparation will probably release a considerable part of the CO_2 in vesicles, whose existence has been recently confirmed[5]. Superimposed on this alteration of mantle ^{40}Ar is the variable contamination with atmospheric Ar[3]. Thus the simple interpretation based on the upper envelope of scattered points seems to be invalid. Further, a basic contradiction follows if we apply their value to a limiting model requiring coherency of ^{40}Ar with K transfer; this coherency must apply in their model to the formation of oceanic crust at the ridges as this is the source of the degassed Ar as well as the transfer of K to the sialic crust (see Fig. 1 of Hart *et al.*[1]). Their derived maximum ^{40}Ar concentration of the mantle source of submarine basalts is a factor of 0.04 lower than the coherent ^{40}Ar concentration computed assuming a K concentration of 0.1%. The coherent concentration represents the ^{40}Ar produced from this K concentration in 4,550 Myr. As I pointed out previously[2], this does not require a single-stage mantle history, only coherent degassing at each melting event. Self-consistency demands use of a computed ^{40}Ar concentration of about 8×10^{-5} ml g^{-1} for the initial magma in a limiting coherent model. Correcting for enrichment on partial melting gives an ^{40}Ar concentration of 2×10^{-5} ml g^{-1} for the depleted upper mantle source. Application of this to an estimate of the K concentration of the non-depleted mantle (their Fig. 3) leads to values of >300 p.p.m. even for very high extent of depletion of the mantle. Their other arguments are not qualitatively changed.

D. W. SCHWARTZMAN

Department of Geology and Geography,
Howard University,
Washington, DC 20059

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HART *ET AL.* REPLY—The concentration of ^{40}Ar in submarine basalts is not critical to the principal observation made in our model calculation of preferential mantle degassing[1]. The main point we stress is the similarity in the ^{40}Ar/^{36}Ar ratio of the atmosphere and non-depleted lower mantle if the atmosphere and sialic crust formed preferentially as a coherent system from the upper mantle. We chose to estimate the ^{40}Ar content of the depleted upper mantle from the ^{40}Ar content of submarine basalts rather than to calculate it indirectly from the K content of the sialic crust and mantle because these values are not well established.

Schwartzman's value of 2×10^{-5} ml g^{-1} for the ^{40}Ar argon content of the mantle is only slightly different from our value of 4.5×10^{-5} ml g^{-1} calculated for the non-depleted lower mantle. Adoption of his calculated value would enhance the similarity in the ^{40}Ar/^{36}Ar ratios of the atmosphere and non-depleted mantle. The concentration of ^{40}Ar measured in submarine basalts is at least an order of magnitude lower than these calculated values. We suggest that this is evidence for an upper mantle depleted in ^{40}Ar relative to the deep mantle and is consistent with the well-established depletion of K in the mantle source of ridge basalt.

There is no evidence that Schwartzman's 'probable significant' degassing of the basalt magma under oceanic ridges by CO_2 bubble transfer is more significant than degassing by basalt–seawater interactions after magma emplacement or than degassing during partial melting and metamorphism in subduction zones and within continental cratons. Because Si and Ca are not partitioned into residual phases or CO_2 bubbles during magmatic processes, the high flux of these elements and ^{3}He observed in geothermal plumes from the Galápagos Spreading Center[2] suggests basalt–seawater interactions rather than bubble transfer are the dominant mechanism for degassing noble gases in ridge basalts today.

We have extensively discussed the effects of atmospheric contamination and degassing on the concentration of ^{40}Ar in submarine basalts and suggest that the surprisingly uniform concentration rules out significant degassing by CO_2 bubble transfer before magma emplacement[3,4]. The ^{40}Ar contents of basalts recovered from depths of over 5,000 m in the Cayman Trough are very similar to those of typical ridge basalts suggesting the ridge basalts have not degassed more than basalts extruded in deep trenches. Basalts with well developed vesicles that have obviously undergone extensive degassing by bubble transfer have been dredged from shallower portions of the ocean and have ^{40}Ar contents up to an order of magnitude lower than the maximum of 3.2×10^{-6} ml g^{-1} we adopted for our model.

ROGER HART
JACK DYMOND
LEWIS HOGAN

School of Oceanography,
Oregon State University,
Corvallis, Oregon 97331

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The late-Würm Arctic ice sheet

I HAVE recently realised that we did not give Dr John Mercer full credit for his earlier contributions when we wrote our paper *Was there a late-Würm Arctic Ice Sheet?*[1]. In our reference to Mercer's paper *A former ice sheet in the Arctic Ocean?*[2] we should have noted that he was the first to make a mass balance calculation for Arctic ice shelves based on present snowfall over the Arctic Ocean and present iceberg calving rates of Antarctic ice shelves; he had also postulated that late-Würm Arctic ice shelves may have occupied the northern Norwegian Sea; and he had also commented on the evidence for ice from the Arctic Ocean moving southward onto the north slope of Alaska. Mercer's (1970) comments on an ice shelf in the Norwegian Sea were a summary of ideas he had developed more fully previously[3].

Our 1977 paper in *Nature* differed from Mercer's (1969 and 1970) papers in that it developed the idea that ice shelves in the Arctic Ocean, Norwegian, Greenland, and Labrador Seas, and Baffin Bay would have been components of a late Würm Arctic Ice Sheet that also included the Laurentide, Innuition, Greenland, Barents-Kara Sea, British, Scandinavian, and possibly East Siberian Sea grounded ice sheets. This connection is implicit, but not explicit, in Mercer's work. We treated the grounded and floating ice complex as a single, unified dynamic system. As a result, our mass balance calculation included not only the Arctic Ocean (which Mercer treated), but also snowfall over the portions of these fringing ice sheets which drained into the Arctic Ocean and its adjacent seas and bays, and distributed that input along an ice-shelf calving front much further south than Mercer's.

TERENCE J. HUGHES

Institute for Quaternary Studies,
University of Maine, Orono,
Maine 04473

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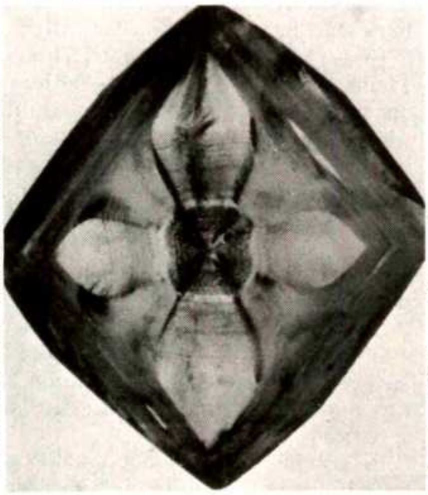
reviews

Diamond facets

John Walker

The Properties of Diamond. Edited by J.E. Field. Pp. 674. (Academic: London and New York, 1979.) £32.50; $67.25.

THIS book could reasonably be subtitled "The Special Research Project: A Second Progress Report", for it chronicles the advances made in diamond research since *The Physical Properties of Diamond* was published in 1965 (Clarendon: Oxford). (This latter should have been subtitled "A First Progress Report", of course.) The Special Research Project was established in 1949 by Sir Ernest Oppenheimer of de Beers and Sir Francis Simon of the Clarendon Laboratory to encourage cooperation in fundamental research between the Diamond Research Laboratory and various British universities (and a very fruitful arrangement it has been).



Central section of a centre-cross diamond (height 5 mm)

From Suzuki and Lang, 1976

What the book lacks in my opinion, and what my subtitles were intended to clarify, is a contribution from Soviet and American scientists. Both of these groups have made considerable advances in pure and applied diamond research. American scientists were the first to publish, in the pages of this journal, a report of the successful synthesis of diamond (Bundy *et al., Nature,* **176**, 51-55; 1955). (Indeed the Americans can not only synthesise diamond; they can do it using peanut butter as the starting material — a valuable discovery if ever the EEC establishes a peanut butter mountain.)

This is not to say that the book does not give credit where credit is due — indeed it does — there are copious references to US and Soviet papers. My point is that the American and Russian authors are in a better position to chronicle their own research, especially the latest developments; they would also bring to bear different ideas and perspectives on current problems in diamond research. There may be difficulties in including contributions from them, but there are precedents.

An integral part of the Special Research Project is the annual Diamond Conference, memorable features of which are the quotations it produces. Dr Field reproduces two favourites. I would like to add two more: Q. "What happens at higher temperatures?" A. "The apparatus melts" (H.M. Rosenberg); "As work in the de Beers Laboratory has shown, the de Beers grit is best" (D. Tabor).

To return to the book, it contains twenty chapters, each written by a different author (or authors), under eight headings: solid state (thermal, optical and electronic properties and impurity content), theoretical, surface (adsorbability and optical properties), mechanical (strength, friction, abrasion, indentation, effects of high temperature, internal structure), physics and technology of growth, geology (of the diamond itself and of inclusions within the diamond) and industrial (abrasive and non-abrasive uses). Finally there is a 12 page appendix which tabulates the physical and chemical properties of diamond, including references to the original research.

The chapter on non-abrasive uses of diamond may contain surprises, even for the diamond specialist. We all know about gemstones, and about cutting and grinding uses. Other uses include: windows (small ones of course!) in satellites; high pressure cells in scientific experiments; microtome knives for eye and brain operations; radiation detectors, especially in radiotherapy; thermometers; and as heatsinks for semiconductor devices.

Although it is unlikely that diamonds will become cheap and plentiful, two chapters in the book suggest potential new sources. While Professor Dawson must be gratified that diamonds are now being found in Australia, the late Professor Tolansky's predictions of diamonds on the moon has yet to be verified. (An interesting statistic is that Tolansky inspected 10 million individual diamonds during his studies — he professed to do it as an alternative to watching television!)

Although Professor Sellschop and his team cannot claim to have investigated so many specimens, they report some fascinating results in their activation analysis studies. The impurity content of diamond has long been a topic of interest, and this new technique has provided data of remarkable quality and quantity — particularly on the submicroscopic inclusions which seem to be droplets of the magma from which the diamonds crystallised. The extension of these nuclear methods to the light elements, which so far have been less intensively investigated, is eagerly awaited, as the light elements are more likely to be able to enter the diamond lattice itself.

Another new technique — cathodoluminescence — is providing a wealth of data about point defects (and linear and planar ones too), as well as elucidating the internal structure of diamonds; it is a valuable complement to X-ray and optical transmission techniques. And one of its practitioners, Dr Lang, seems likely to replace Professor Tolansky as a supplier of beautiful photographs of diamond.

Optical micrograph of etched near-central section (2.4 mm square) through natural octahedral diamond

From Harrison and Tolansky, *Proc. R. Soc.,* 1964

It is impossible in the space available to consider in detail all 20 chapters of the book; suffice it to say that the authors are leading lights in their fields.

One of the surprising things about diamond — which in principle is one of the simplest solids one could envisage — is the wealth of studies it has generated, and the continuing exhibition of new and interesting features. Yet we still do not know how it was generated by nature, nor do we understand its frictional behaviour, despite the manifest importance of such topics. But we are trying hard, as the 674 pages of this authoritative book indicate; this is where a scientist must start if he is interested in any facet of diamond. □

John Walker was a Research Fellow at the Universities of Reading and Paris, studying the optical properties of diamond. He is now a microprocessor consultant.

Earthquake phenomena

The Earthquake Handbook. By Peter Verney. Pp. 224. (Paddington: New York and London, 1979). £5.50.

The Earthquake Handbook is an excellent description of earthquake phenomena and fulfills the objectives set out by the author in the introduction: "This is not a technical book: those wanting detailed scientific information [on earthquakes] should consult some of the many excellent works available . . . My interest has been to explain this extraordinary natural phenomenon, referring to the records of past earthquakes, and in the light of modern progress in seismology, to help those who may live in or visit one of the many earthquake-prone areas of the world". The extensive quotations from eye-witnesses to the earthquakes described and the opinions of their contemporaries are certainly the outstanding features in this book, but the author's own contributions are clear and concise. The book is illustrated by an excellent selection of photographs, prints and drawings, all well reproduced. The text, figures, and quotations taken together give as good a description of what an earthquake is really like as any other source short of the earthquake experience itself. However, it should be understood that the treatment is descriptive and the technical aspects of seismology are scarcely touched on. In this sense the title of the book may have been poorly chosen.

The book begins with descriptions of the 1906 San Francisco and 1755 Lisbon earthquakes. These descriptions are amplified in subsequent sections of the book, together with a description of the 1923 Tokyo earthquake based principally upon the eye-witness account of H.W. Kinney, an author and editor who was travelling near Tokyo at the time. Shorter descriptions of the earthquakes at Port Royal, Jamaica (1692), London (1750), Calabria (1783), Messina (1908), Agadir (1960), Skopje (1963) and Alaska (1964) are also included.

The book then gives a very brief account of the various earthquake theories from the earliest mythologies (which generally attributed earthquakes to movement of the animal that was thought to support the Earth) to Aristotle (winds blowing through subterranean passages), Lucretius (rocks displaced in subterranean caverns), Ben Franklin (an electric discharge from a cloud to Earth), and John Michell in 1760 ("earthquakes were waves set up by the shifting masses of rock miles below the surface". Professor Koto of Japan in 1891 was apparently the first to assert that faulting was the cause (and not the effect) of the earthquake. Verney, of course, quotes extensively from those who attribute earthquakes to divine punishment (for example, Royal Society, 1752, "earthquakes only occur when people need chastening"). The section concludes with an elementary description of plate tectonics.

A description of earthquake geography and earthquake effects follows. The latter section is particularly concerned with tsunami (tidal waves generated by earthquakes) but includes discussions of earthquake noises and lights as well as the physiological shock experienced by earthquake survivors and even the motion sickness reported by some.

The basic rules for survival in an earthquake are given in a brief but practical chapter that includes advice on how to prepare for a possible earthquake as well as how to behave during and after the shock. The final three chapters are brief, non-technical treatments of earthquake engineering, prediction, and control. I believe most scientists will find these final three chapters somewhat superficial.

The book is clearly intended for a lay audience, but I believe even seismologists will find the many quotations and general descriptions of real interest. However, the prospective reader is reminded of the author's admonition "This is not a technical book . . .". **James C. Savage**

James C. Savage is a geophysicist in the Office of Earthquake Research, US Geological Survey, Menlo Park, California.

Nutrition and national development

Food and Nutrition Policy in a Changing World. By J. Mayer and J. Dwyer. Pp.300. (Oxford University Press: Oxford and New York, 1979.) £8.25.

LIKE many books which are edited rather than authored this slim volume seems to me to lack the sense of coherence and direction that would seem a *sine qua non* for it to achieve its aim of dissusing the need for placing food and nutrition in the larger context of national development. This failing is doubly said, as it is not merely a book, but a condensation of a five-volume report commissioned from the editors by UNICEF in order to "set forth goals for UNICEF and allied agencies for the next decade".

What use the report will be to UNICEF, only time will tell; I found the book unsatisfactory to read. Partly this was no doubt due to the level at which it was pitched, for as the editors warn: "The specialist would regard the book as nontechnical and somewhat cursory". But it was more than that: it was a sense that it was not a book at all but a collection of disparate chapters loosely edited and fitting together into a scheme which seemed only to exist as the various subheadings in the table of contents.

This is not to say that some of the individual chapters were not quite enjoyable as discrete essays: the editors themselves contributed a chapter entitled "Beyond Economics and Nutrition: The Complex Basis of Food Policy" which was a reprint of their polished critique of approaches to control of malnutrition, first published in *Science*. Some other chapters, notably those by Perisse and Ms Jeliffe, were also well written expositions of the views of the authors. But in this I found the flaw with the book as a whole. Each author seemed to perform a solo item, without reference to his colleagues so that overall the book did not hang together.

I was irritated by such contraditions as Kimm and Donoso repudiating the idea of dietary protein deficiency on page 160, while Winikoff invoked it *en passant* on page 184; or again that Winikoff's chapter advocating megadoses of vitamin A as an effective treatment for vitamin A deficiency was followed by an editorial note pointing out it was a high risk and probably ineffective strategy. Besides such tactical differences, there seemed also to be a difference in grand strategy between the advocates of economic intervention and those who felt that health care was specifically needed.

In general, I would not criticise any scientific work for the divergencies in views it contains. After all, without contradictions there is no science; the great mistakes in nutrition have arisen from professional certainty, not doubt. But the differences in view in this book are not productive ones. There is no air of creative tension between them; on the contrary, the overwhelming impression is that the authors have not even discussed their views with each other. I do not know why this has arisen, I suspect because of a lack of firm editorial control at the centre, and perhaps for some authors a lack of balanced scholarship in the area which they seek to discuss, but it is difficult to judge the extent of scholarship in the space allotted to each author. The editors would have done better to throw out some topics completely (for example, Dr Araujo's ludicrously terse "Nutrition Primer") and enlarge other issues in depth. But they did not, and I suspect the result is a book that will not interest the layman or satisfy the specialist.

John Rivers

John Rivers is Lecturer in Human Nutrition at the London School of Hygiene and Tropical Medicine, London, UK.

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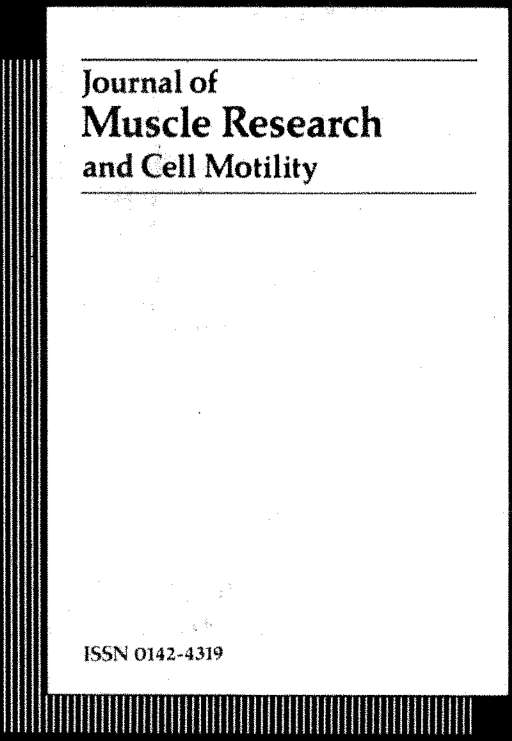

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HLA system

The HLA System: An Introductory Survey. Second edition. By A. Svejgaard, M. Hauge, C. Jersild, P. Platz, L.P. Ryder, L. Staub Nielsen and M. Thomsen. Pp. 111. (S. Karger: Basel, London and New York, 1979.) DM 43; $21.75; SFr. 36.

THE HLA system of tissue antigens is acquiring a growing importance in the study of cellular interactions. A coherent, lucid account such as this volume attempts is to be welcomed. The small size of the book (111 pages) in no way reflects a paucity of information. The authors have achieved the combination of brevity and clarity, at the same time providing useful links for readers who may come to the subject from disciplines which do not encompass genetics or human disease.

The scope of work now relevant to HLA is indicated by the contents page, with topics ranging from immune response (Ir) genes to complement, transplantation, transfusion, pregnancy, paternity testing and disease association. The bibliography is comprehensive and commendably up to date. There is a simple but adequate account of the biology of the HLA system, with particular reference to the rôle of the major histocompatibility complex (MHC) in the immune response. This section also includes a summary of current thinking about the specific role of HLA antigens as cell membrane products (? receptors).

There is a good appraisal of the current status of HLA in clinical medicine, including both transplant matching and disease association studies. The authors have summarised these two major applications and give a very honest view of the advantages and disadvantages of applying HLA studies in these areas. There is a short concluding section on methods in HLA typing which is more than adequate for those who may not work directly on HLA but need to know about the general techniques.

This book could be required reading for all non-HLA workers who are proposing to collaborate on HLA studies with the experts in the field. It would save a great deal of time presently spent on lecturing on HLA to various groups of medical and scientific workers who are looking for a simple, understandable introduction to HLA.

Heather M. Dick

Heather M. Dick is Consultant in Clinical Immunology at Glasgow Royal Infirmary, Glasgow, UK.

Physics of neutron stars

Neutron Stars. By J.M. Irvine. Pp.155. (Oxford University Press: Oxford, 1978.) £7.95.

IRVINE'S monograph is the first book devoted to the physics of neutron stars, the second most extreme endpoint of stellar evolution. The primary content is an informal review of the theory of neutron star structure, with emphasis on our understanding of the physical processes in condensed matter that is needed in order to construct 'realistic' models.

The book begins with a broad outline of stellar evolution, and the mass-radius relationship of neutron stars is estimated in the manner familiar to students of stellar interiors since the work of Eddington. The basic observation of pulses from isolated pulsars is then briefly described, followed by a repetition of the now classic arguments for the identification of pulsars as rotating neutron stars, based on their rate of increase of pulsar periods and the shortness of the period of the Crab pulsar. (The use of the Crab nebula as a box calorimeter to measure the moment of inertia of the central object, an equally crucial part of the identification, is not mentioned.) This is followed by a fairly detailed discussion of glitches in the spindown of the Crab and Vela pulsars along with a review of the starquake model. No mention is made of the 'restless' random walk of the rotation frequency of the Crab pulsar, which is probably also present in the Vela and many other pulsars.

From here, Irvine turns to an outline of cooling processes that can influence the final temperature of a neutron star, both in the deep interior and at the surface. He does a reasonably good job of outlining the significance of superfluidity, superconductivity and the formation of a pion condensate for the cooling rates, a topic which is rapidly increasing in experimental importance as the HEAO-2 observing programme moves forward. An understanding of his discussion requires at least slight acquaintance with the BCS theory of superconductivity. He then turns, in chapter 4, to a brief discussion of neutron star "exteriors". Here, the magnetosphere is treated solely as a source of vacuum magnetic dipole radiation (including the ~ 20% relativistic corrections!), in spite of his mentioning the requirement of there being an electrically significant number of charges in the environs. Irvine then returns to the less controversial realm of condensed matter by describing the peculiar atoms and solids which make up the perfect lattice (density ~ 10^4 g cm^3) expected to form the very outermost layers. This region (often called 'Ruderman's whiskers') is treated with about the same depth but less clarity as is contained in Ruderman's discussion in the

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IAU Symposium No. 53 (D. Reidel: Dordrecht, 1974, p117). A few remarks are made on possible radiation of pulsed photons by charges accelerated along polar field lines, including some incorrect remarks on the formation of purely radio pulses because of synchrotron self-absorption of higher emitted frequencies.

The topic of observational appearance is then rapidly abandoned for a return to the interior, with a brief outline of the equation of state including various possibilities in the very high density region (pion condensation, hadronic excited states and cores, and "quark soup"). The application of these equations of state to the calculation of stellar models and the maximum and minimum masses of neutron stars is then outlined, and the book concludes with three brief appendices on methods for calculating the correlations which give rise to superfluidity, as well as a few results from general relativity needed to obtain the 20-30% corrections of Newtonian theory expected for these objects.

Although this book will be mildly useful to specialists in neutron star physics (and somewhat less than mildly useful to those concerned with pulsars as radiating bodies), it suffers from a number of serious problems. It is very badly organised; topics in condensed matter physics are interspersed with astronomical issues (such as scintillation of pulsar radio signals) and with occasional excursions into rudimentary discussion of the magnetosphere. Apparently, the text was written before 1976 and has not been substantially updated; for example, the thick crust neutron stars of Pandharipande, Pines and Smith are not mentioned, and pulsed accreting X-ray sources do not appear at all. This latter omission is especially astonishing, as our real laboratory of neutron star interiors is likely to be the accreting objects in binary systems, not the ". . . vast amount of information . . . buried in the glitch phenomena . . ." of isolated radio pulsars.

Irvine's discussion of the coupling of the isolated neutron star to the outer world is so perfunctory that its omission would have improved the book. For example, I find it difficult to understand the inclusion of the calculation of vacuum dipole radiation in curved spacetime (a 20% correction), while no illustration is given of the orders of magnitude uncertainty introduced by our lack of understanding of the exterior plasma configuration. His discussion of how pulses form is wholly out of touch with modern ideas; however, given the lack of contact between these ideas and the observational details, this topic would also have been better omitted, or dealt with in sufficient depth to illustrate the nature of the problem. His discussion of the interior physics is adequate for the expert, but inadequate as an educational tool for those not already acquainted with theoretical many body physics.

As a reference source, the book is incomplete, and not more up to date than the thorough review by Baym and Pethick (*A. Rev. nucl. Sci.*, **25**, 27; 1975). The flavour of *Neutron Stars* is that of unrevised, rather outdated lecture notes for an advanced course, without the compensation of new ideas or a unified point of view. A monograph which is truly useful remains to be written. **J. Arons**

J. Arons is Professor of Astronomy at the Leuschner Observatory, University of California at Berkeley.

Individual and group differences

The Psychology of Individual and Group Differences. By L. Willerman. Pp.531. (Freeman: San Francisco and Reading, UK, 1979.) Hardback $15; £8.70.
Readings About Individual and Group Differences. Edited by L. Willerman and R.G. Turner. Pp.330. (Freeman: San Francisco and Reading, UK, 1979.) Hardback $14; £8.90; paperback $6.50; £3.80.

THE analysis of individual and group differences, notably those connected with one form or another of ability, has become enmeshed in the confusions of political ideology. Theories that human talents may be inherited, or even that there are human talents, have fallen under a threat of popular disapproval divorced from the scrutiny of evidence or of academic discourse. Given the intolerant tyrannies of the past it is not so hard to interpret the censorship of Copernicus or the curtain drawn over the free development of genetics in Stalin's Russia. But this new censorship, arising from outside the formal apparatus of the state, is more difficult to account for, although in character and effect it is no less insidious. As a consequence, in recent times, academic thinkers willing to argue for the inheritance of intellectual skills have been subjected to strong attacks, often of an unreasoned kind, and their views have been identified with common prejudice. It has sometimes required an act of courage to permit open consideration of the empirical evidence.

The author of this new text on the psychology of individual and group differences pays scant attention to such distractions, and leads the reader straight into the heart of his subject. His report is directed unambiguously to a hierarchical conception of difference. The first chapter deals with the academic background of research, wholly ignoring political overtones or implications. The next two cover some basic elements of statistics and genetics thought necessary for an understanding of the later sections. Inevitably these are brief and probably too difficult for the complete beginner in either discipline, but they do serve as a tribute to the need to master such attendant skills. If no more, they alert the reader to think seriously about the methodological problems that have to be tackled in psychological research.

In the second and major section of the book, the author works patiently through some of the main topics in the current psychology of individual difference: intelligence, genius and their evil counterpart, mental retardation; educational achievement; personality; psychopathology and criminality. Systematically he tackles three basic questions in turn: what is the conceptual status of the difference(s) involved; how far differences are genetically determined; and how far they are environmentally conditioned. At each point the extensive survey of evidence and research is made more valuable because of the careful and critical attention devoted to methodological aspects in the studies reviewed. There is a similarly critical and thorough treatment in the final section which is concerned with three types of group difference: those based on sex, age and race.

In any general text there are bound to be omissions. Here the discussion of occupational and career differences is narrow in scope, excluding a whole range of studies into attitude and performance. There is no reference to the main body of Kohlberg's research into moral differences, or to the investigation of differences in values. The assessment of variety in the forms of excellence is also limited: the work of Piaget, for instance, occupies but two pages, and interesting research by Hudson and others on divergent thinking is altogether ignored. It is perhaps surprising, too, that in the section on group differences there is no separate chapter on social class, and the implications of Bernstein's enquiries into language do not receive appropriate attention in the relatively casual account of conclusions reached by Hess and Shipman. Nevertheless, this book generally provides a scholarly and dispassionate review of a massive research field, and does so succinctly and clearly. It may be recommended to the intending student and teacher of psychology, and also offers the interested lay reader material that is both readable and substantial.

The companion volume of readings is well selected and appropriate to the text. Contents have been skillfully edited to limit the inclusion of spurious material and, in company with the text, they serve to illustrate both the conclusions of research and some of the inherent methodological preoccupations. **John A. Glossop**

John A. Glossop is Lecturer in Education in the School of Education, University of Leeds, UK.

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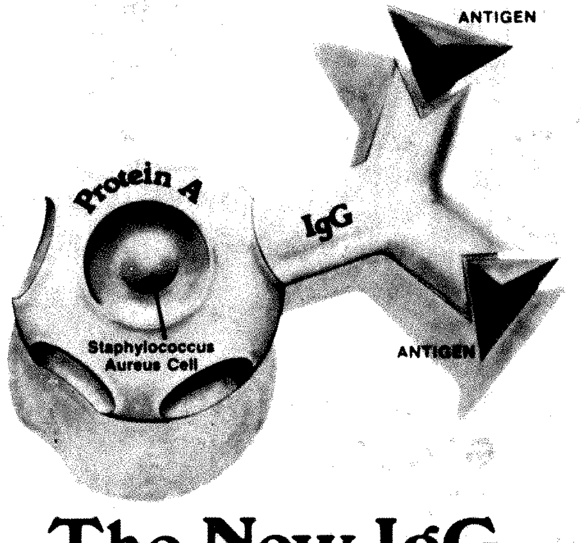

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obituary

Arend Bouhuys

Arend Bouhuys died on board the *Queen Elizabeth 2*, of a sudden and unexpected heart attack, on 15 June 1979, at the early age of 53. He had resigned from his appointment at Yale University and was on his way to Europe to become chairman and professor of the Department of Physiology at the University of Utrecht in his native country.

His untimely death has deprived occupational medicine and respiratory physiology of an active and brilliant research worker who had made an outstanding contribution to environmental lung disease and, in particular, byssinosis or brown-lung disease suffered by textile workers.

Bouhuys was born in Deventer, in The Netherlands, in 1925. He graduated MD at Utrecht in 1948. He spent his early years as a resident in internal medicine and chest diseases and as an instructor in physiology. He soon developed a special interest in pulmonary disease and methods of measuring lung function. At the age of 29 he was appointed head of the Pulmonary Function Laboratory at the University of Amsterdam. He had by then become interested in byssinosis and in 1959 he published the first of a series of original papers on effects of cotton, flax, and hemp dust exposure on respiratory symptoms and lung function.

In 1962 he left The Netherlands to take up an appointment in Emory University School of Medicine in Atlanta, Georgia. Two years later he was made a Fellow of the John B. Pierce Foundation in New Haven and was offered an associate professorship at Yale University where, in 1968, he was appointed full Professor of Medicine and Epidemiology. During his 15 years at Yale his output was prolific. He published 86 original research papers, two books on the physiology of breathing and lung disease and numerous review articles and abstracts. In 1975 he became managing editor of *Lung* which, under his direction, attracted good papers and increased its circulation.

By using computers for recording and analysing respiratory symptoms (by questionnaires) and lung function, he developed sensitive and accurate methods of measuring acute and permanent changes in lung function from exposure to air pollutants. In this way he advanced epidemiological techniques for investigating chronic respiratory disease in communities. His most ambitious study, undertaken by a multidisciplinary team, was of 8,000 community residents in urban and rural districts in Connecticut and a rural population in South Carolina.

Two of his most valuable papers were published a year before he died. One gave new equations based on height, age and weight for predicting normal lung function values in healthy blacks and whites. The other paper was published in *Nature* **276**, 466-471, (1978). It produced evidence from his own and other studies that cigarette smoking and textile dust exposure were far more deleterious to health than out-door air pollution. He concluded that no measurable effects could be expected from further air pollution control with respect to prevalence of chronic bronchitis, asthma and loss of lung function, but, instead, there should be systematic efforts to prevent cigarette smoking and to control exposures to textile dusts and other inhalant risks at work.

In Utrecht he planned to continue research into isolating the causal agent of byssinosis.

If Arend Bouhuys had not been strongly motiviated to apply his considerable skills and energy to the prevention of lung disease, he would have remained a laboratory research worker. Instead, with his co-research workers in various parts of the world, he undertook field studies of textile workers in several countries, including Spain, Holland and the United States. In the U.S. he focussed attention on brown-lung disease, which had been overlooked. His research findings and his own personal commitment helped to establish strict occupational health requirements for US cotton workers through dust control and medical surveillance.

He is survived by his wife, Fenna, who helped him a great deal in his work, particularly in preparing papers; a son and four daughters, two by a former marriage.

R.S.F. Schilling

P.C. Caldwell

The Death of Professor P.C. Caldwell FRS, on 6 June 1979 at the age of 52 saddened his many friends and came while he was still at the height of his powers.

He was born in Warrington and belonged to a family which had lived there for several hundred years and prospered despite suffering heavy penalties for its steadfastness in the Roman Catholic faith. He was sent to Ampleforth School and there his exceptional academic gifts were recognised but these were so varied that the choice of career proved difficult. He finally decided on a scientific career and went as a scholar to Trinity College, Oxford where he studied physical chemistry for his first degree.

After graduating he stayed in Oxford to do research with Sir Cyril Hinshelwood on nucleic acid in bacteria. Together they showed that DNA per cell mass was related to the rate of growth, and hence protein synthesis, and discussed the need for a relationship between nucleic acid and protein, suggesting that two nucleotides would specify one amino acid. This was the first discussion of the coding problem.

From Oxford Peter Caldwell went to work with Professor A.V. Hill and Sir Bernard Katz at University College, London. It was there that be began his main life's work on the application of physico-chemical methods to biological problems. He made the first successful experiments in which micro-electrodes were used to measure intracellular *p*H. This led to a whole field of study in which similar electrodes are used to measure intracellular ion activities *in vivo*. In the course of this work he made the useful discovery that the leg muscles of the crab *Maia squinado* consisted of very large fibres 1-2 mm in diameter.

A.V. Hill believed very strongly that physicists and chemists entering biophysics should be sent to a marine biological station and so exposed to a great range of biological material and problems. It was not surprising, therefore, that Caldwell moved next to the Marine Biological Association's Laboratory in Plymouth and there, and at Bristol University, he continued to make key experiments in difficult and very competitive fields of study, often inventing powerful new techniques. With Sir Alan Hodgkin, Professor R.D. Keynes and other colleagues he played a leading role in the spectacular post-war advances in our knowledge of nerve and muscle.

Among the results of his many studies were: the first determinations of ATP, arginine phosphate and orthophosphate in single cells; providing the first example of a spatially orientated enzyme in a living membrane showing different sensitivities to inhibition on its two sides; and finding, on single crab muscle fibres, the first evidence that *ionised* calcium exists in very low concentrations in cells and that cell activity can be triggered by an increase in this calcium level. At Bristol University he trained a series of postgraduates in research and, with them, made important progress in our knowledge of the influx and efflux of substances from living cells. He was elected FRS in 1975.

Caldwell had many interests to which he gave himself wholeheartedly and in all of these his knowledge was deep and his technical skill so high that he could deal with acknowledged experts on equal terms. An excellent pianist, he was devoted to music and composed about 30 piano sonatas. It was in accord with his character that he was especially attracted by the work of composers who, like Schubert, took a theme and followed it through many variations.

Peter Caldwell's exceptionally gentle and kind personality made him much appreciated by a wide circle of friends. When relaxed he was most entertaining company and he was always helpful in the lives and work of his colleagues and students. He will be widely missed and our deepest sympathy goes to Mrs P.A. Caldwell and their children Helen, Margaret, Philip, Anne and Thomas.

E.J. Denton

John Jaeger

PROFESSOR JOHN CONRAD JAEGER, Emeritus Professor of Geophysics in the Australian National University, died on 15 May 1979 after a long illness, at the age of 71.

Born in Sydney on 30 July 1907, he was educated at the Church of England Grammar School, Sydney, and at the University of Sydney from which he graduated Bachelor of Science in 1928 with first class honours and university medals in mathematics and physics. In that year he went to Cambridge University where he received first class honours in the Mathematical Tripos. Following five years of research at Cambridge in theoretical physics, he joined the University of Tasmania in 1936 as Lecturer in Mathematics, subsequently becoming Professor of Applied Mathematics in that University. In 1942 he was awarded the degree of Doctor of Science in the University of Sydney.

Shortly after the founding of the Australian National University, John Jaeger was invited to accept a Chair and to set up a Department in the Research School of Physical Sciences. He took up his appointment as Professor and Head of the Department of Geophysics in 1952, and remained in that post until his retirement in 1972. Under his direction, the Department of Geophysics developed rapidly into one of the leading research groups of its kind in the world. In 1965, it became the Department of Geophysics and Geochemistry and more recently (1973), was reconstituted to form the Research School of Earth Sciences, at least in part in recognition of the high international reputation that the department established under John Jaeger's leadership.

The direction of development of the deparment was largely based upon his strongly held belief that the application of the basic sciences of mathematics, physics and chemistry, in concert with more conventional geological methods, to the study of the earth, would result in major advances in our understanding of the origin and history of the earth. His recognition of the importance of interdisciplinary fields, together with his ability to attract staff and students, were major factors in establishment of the high reputation of the department, both nationally and internationally, in a relatively short period.

John Jaeger had a most distinguished career as mathematician, physicist and geophysicist. His scientific contributions covered an extraordinarily diverse range of interests, including theoretical physics, radiophysics, ionospheric and solar physics and meteorology, but the main emphasis of his research was in the application of mathematical techniques to a wide variety of theoretical and practical problems, especially the conduction of heat. On establishing the Department of Geophysics in the Australian National University he embarked upon vigorous experimental programmes concerned with heat flow in the Australian continent, and the behaviour of rocks under stress (rock mechanics). John Jaeger was a man of great scholastic achievement; he published more than 130 articles in the scientific literature, and was author or coauthor of six books in applied mathematics and rock mechanics, which are regarded as standard works in their fields. His book (with Carslaw) *The Conduction of Heat in Solids* is widely accepted as a classic in this area.

In recognition of his achievements, Jaeger, in 1954, was elected a Fellow of the Australian Academy of Science, of which body he was Vice-President in 1958-1959, and in 1970 he was elected Fellow of the Royal Society (London). He was also Doctor of Science (*honoris causa*) of the University of Tasmania. In 1971, he was awarded the Rankine Lecture by the Institution of Civil Engineers (London).

John Jaeger was a big man, both physically and mentally. He was a man of great humanity but basically very shy. He never sought the limelight and was extremely modest about his own achievements. He set high standards for himself and expected staff and students alike to strive for excellence. His influence in many fields, including the earth sciences, in Australia and indeed in the world, has been profound. He will be sorely missed.

Ian McDougall

J.B. Birks

DR JOHN B. BIRKS, reader in physics at Manchester University died suddenly on 1 March 1979, a few months after the death of his wife Margaret in a car accident. He leaves a son and daughter.

He was born on 6 March 1920. After graduation from Oxford in 1940 he joined the Telecommunications Research Establishment for the duration of the war. His work there on radar led to his work on microwave absorption in ferrites, when he joined the Department of Natural Philosophy at Glasgow and for which he received his PhD in 1947. An interest in scintillation counters led him into the field of scintillators and the photophysics of organic molecules which became his main scientific interest.

In 1951 he became professor of physics at Rhodes University, South Africa, where he quickly established an active research group. However, his dislike of the developing racial policies made him return to Britain in 1954 as diretor of research at British Dielectric Research. In 1957 he moved to Manchester to resume his work on photophysics and in a few years had built up a large and thriving research group which later, with his encouragement, diversified into electron scattering, lasers, microwave spectroscopy, vacuum ultraviolet spectroscopy and latterly, polymer physics.

An empiricist, he was probably most gifted in correlating the mass of data in the field and extracting the essence. He had a good physical insight and made substantial contributions to his subject. A prolific and fluent writer he published some 180 papers, edited the proceedings of many conferences, e.g. the Rutherford Jubilee International Conference 1961, and the series *Progress in Dielectrics*. He is probably best known for his books *Scintillation Counters* (1953), *Theory and Practice of Scintillation Counting* (1964), *Photophysics of Organic Molecules* (1970) and the two volumes of *Organic Molecular Photophysics* (1974). Several monographs on scintillation counting were widely distributed by several companies in the field. He was a consultant to many companies and national laboratories, in demand as a seminar speaker and a great attender of conferences where he met his many friends who will now sadly miss his stimulating presence. An enthusiast for his subject he was on occasions guilty of going a bit over the top but his friends would readily forgive him.

John Birks was a man of wide culture and liberal humanistic views, and was always prepared to defend what he thought was right and to support his friends. Never an establishment man he clashed with authority on several occasions which possibly affected his career. A most efficient and capable administrator, and an excellent teacher and researcher he had much to offer.

He was a generous host who entertained well and was particularly kind to his students, many of whom came from overseas. He made them feel very much at home, and now, spread around the world, they will be saddened by his death.

The loss of his wife was a great blow. They will both be greatly missed by his son and daughter and their families. I am glad that for 28 years I knew him both as friend and colleague.

Scott Hamilton

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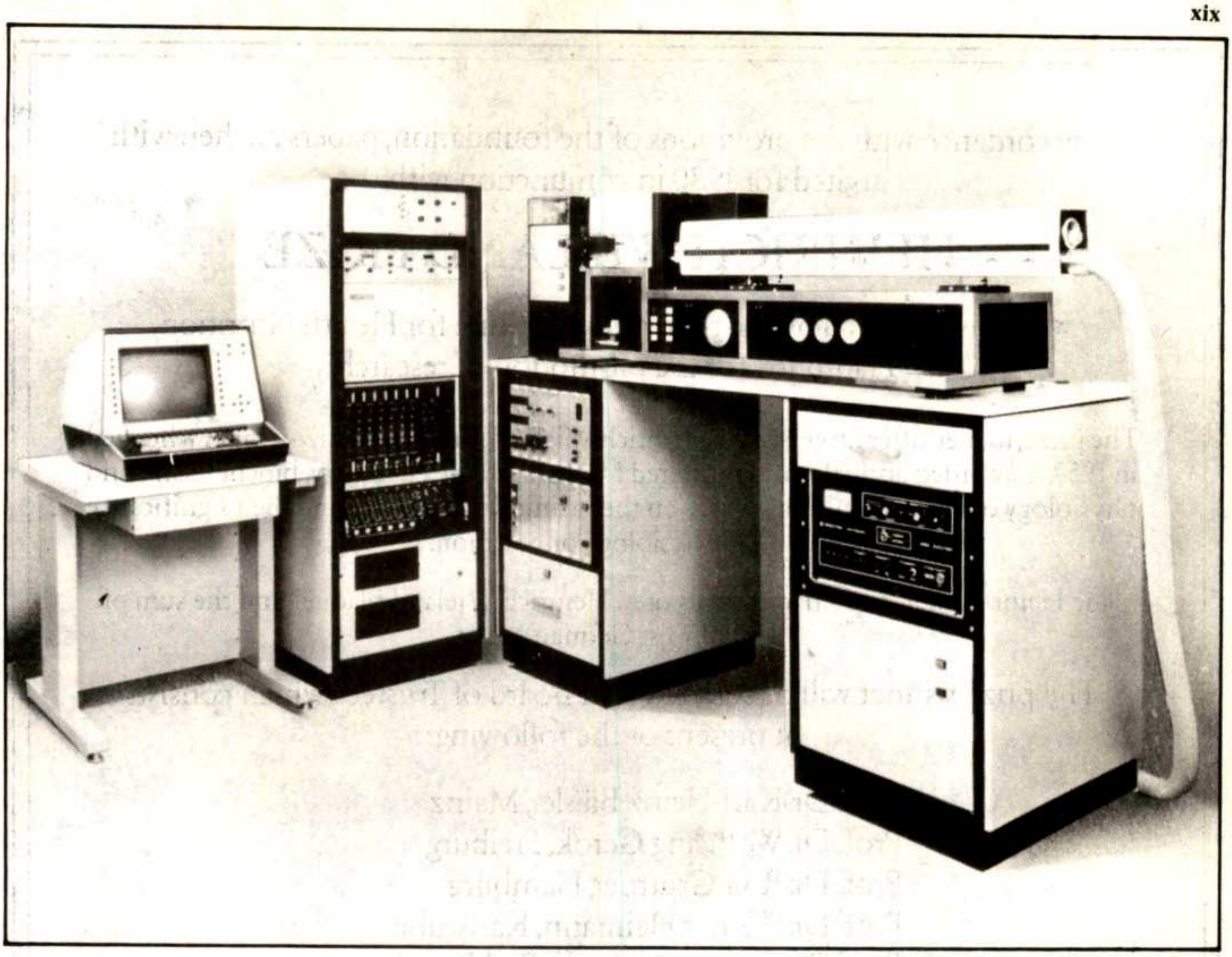

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In accordance with the provisions of the foundation, papers are herewith invited for 1980 in conjunction with the

HEINRICH WIELAND PRIZE

which is endowed by the Margarine Institute for Health Nutrition, Hamburg, for the promotion of research.

The prize, named after Professor Dr. Heinrich Wieland, the Nobel Prize winner who died in 1957, is awarded annually and is offered for work on the chemistry, biochemistry and physiology of fats and lipids, as well as on their clinical importance and their significance in the physiology of nutrition.

The Heinrich Wieland Prize consists of a „Heinrich Wieland plaque" and the sum of 15,000 West German Marks.

The prize-winner will be selected by a Board of Trustees which consists at present of the following:

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Persons eligible for the HEINRICH WIELAND PRIZE for 1980 are authors of unpublished scientific treatises or treatises published during the period from 1979 to 1980. Papers must be written in German, English or French. All Papers in English or French must be accompanied by a summary of about 3 pages in German. Treatises that have already been awarded some other prize for scientific work are not eligible.

The closing date for sending in Papers for the 1980 award is 1st March, 1980.

A single copy of the Paper must be sent by this date to the following address:
Board of Trustees for the Award of the Heinrich Wieland Prize
Prof. Dr. Alfons Fricker, Ringelberghohl 12, 7500 Karlsruhe 41, West Germany.

The presentation of this year's prize will take place at 10.30 a.m. on 12th October, 1979 in the Adolf-von-Baeyer Lecture Hall of the University of Munich, which is located in Meiserstrasse.

Circle No. 27 on Reader Enquiry Card.

new products: RIA and electrophoresis

These notes, prepared from material provided by the manufacturers, are intended to give an outline of the range of products on the market. Information on further equipment can be found in the insert in the centre of the journal. For more details use the Reader Enquiry Card bound inside the cover.

A feature on laboratory instrumentation will appear in the 22 November issue.

COLLABORATIVE RESEARCH INC.

To complement the established RIA systems for the measurement of cyclic AMP and cyclic GMP, Collaborative Research Inc. have now introduced an RIA system for the detection of cyclic CMP. The Collaborative Research cyclic CMP RIA system is sensitive in the femtomolar range and provides high CMP antigen, cyclic CMP standard, reagents for acetylation and rabbit γ-globulin. These components may also be purchased separately.

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A.R. HORWELL

Diagnostic Products of Los Angeles have recently added several new RIA kits to their range. These include kits for the assay of aminoglycoside antibiotics netilimicin and sisomicin, as well as testosterone, LH and FSH. These kits possess high sensitivity and specificity combined with low cross-reactivity.

Other new items of interest to laboratories performing RIA techniques are the special heads available on the Sigma 2 and 3 range of centrifuges. These heads have been purpose-designed to cope with the large numbers of tubes used in these assays. The heads are swing-out type; that for the Sigma 2 will hold 20 tubes and that for the Sigma 3 up to 100 tubes. Both centrifuges are available with refrigeration if required; the Sigma 2K refrigerated model, for example, is a compact bench-top model operating down to −10°C.

The Buchler vortex evaporator has proved itself invaluable in laboratories processing large numbers of tubes for RIA. The instrument has facilities for vortexing aluminium blocks containing numbers of tubes, with or without simultaneous vacuum and with either heating or cooling. The heating and vacuum facilities allow for the most rapid possible evaporation of liquids if required. Test tube blocks are available for a variety of tubes, e.g. one to take 90 x 12 mm tubes, or one for 27 scintillation vials.

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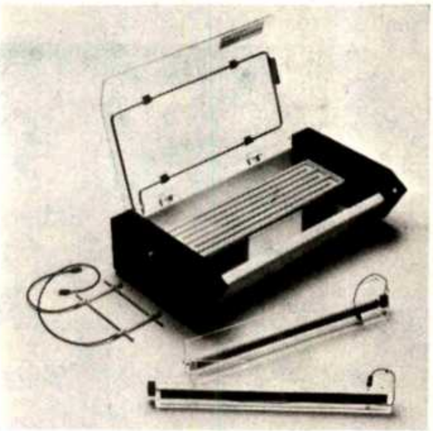
BIO-RAD

The versatile Model 1415 electrofocusing/electrophoresis cell from Bio-Rad is the basic unit in five complete systems for high resolution analytical and preparative electrofocusing, immunoelectrophoresis, zone electrophoresis and preparative DNA separation. Advanced design features of the cell include a large, rectangular cooling platform (125 × 430 mm), a condensation control coil that keeps moisture off lid and gels and dual electrode jacks for running two electrofocusing experiments at once. The platinum ribbon electrofocusing electrodes are detachable, adjustable and are available in two sizes for maximum flexibility in experimental design. The immunoelectrophoresis/electrophoresis electrodes are built into removable buffer chambers that are easily emptied and cleaned. Reagents and accessories can be added as needed for each application, and are available in five convenient applications kits. Many accessories present new designs for substantially improved performance.



The Bio-Rad Model 1415 for flat bed electrofocusing

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ISCO

The ISCO model 1310 gel scanner transports an electrophoresed gel through the optical unit of an ISCO model UA5 UV monitor, recording UV absorbance of unstained gels or visible wavelength absorbance of stained gels. UV scanning of a cylindrical gel in its original quartz running tube simplifies determination of the minimum time required for maximum resolution and provides accurate quantitation of each peak. Subsequent staining and visible scanning of the same gel then offer increased sensitivity and resolution. Slab gels as well as rod gels can be scanned as can gels affixed to Mylar strips. Scan speed is either 30 or 150 cm per hour and these speeds can be matched up to the recorder speed so that if desired, the gel can be laid on the chart to easily locate zones. UV scanning eliminates the need to remove gels from their running tubes for staining and destaining. The model 1310 will take 25-cm gels which are very inconvenient to stain and scan conventionally because of difficulties in removing them from electrophoresis tubes. ISCO quartz tubes have a wall of 6 mm and tolerances on both inside and outside diameters are held to 0.001 of an inch to assure accurate quantitation. The ISCO scanner can transport two gels simultaneously through the measuring and reference lightpaths of the absorbance monitor. This technique is extremely useful for reducing background empholyte absorbance when scanning isoelectrically focused gels. The ISCO gel scanner is the ideal instrument to use in those biochemical research laboratories which have a column chromatography workload, which would enable them to use the model UA5 monitor, both as a UV detector and as a gel scanner.

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UNIVERSAL SCIENTIFIC

The compact Universal Scientific Uniscil/Gradipore polyacrylamide gel electrophoresis system is one of the most versatile systems available. Each of the two electrophoresis tanks will accept both rod and slab gels enabling virtually all types of PAGE to be performed. The running tanks incorporate as standard, a buffer circulation and cooling system. Unique to Uniscil are the Gradipore gels. These ready to use pre-cast slab gels contain a continuous polyacrylamide concentration gradient between a variety of concentration limits. The gels achieve separation of macromolecules by molecular sieving in the molecular weight range 3,000,000 to 30,000 to give high resolution. The gels can be used to accept up to 14 samples each for single dimensional running or they can be used as the second of many two dimensional techniques, including isoelectric focusing. The Uniscil electrophoresis range also includes power packs, electrophoretic destainers, gel casting apparatus, gel slicers, and micropreparative attachments.

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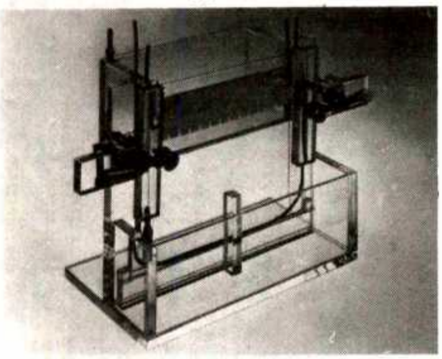
ATLANTIS SCIENTIFIC

The Atlantis Model HS horizontal submarine gel electrophoresis chamber provides a simple low-cost apparatus for the electrophoresis of most macromolecular substances including DNA, RNA, and proteins. With the gel medium immersed in the buffer solution, the horizontal submarine electrophoresis technique evenly dissipates heat and eliminates hot spots. Combined with the 11-inch long gel tray, the result is clear separation of bands and better resolution than is normally achieved with conventional techniqúes. The system is ideally suited to separation in agarose gels, and may be used preparatively or analytically. Both glass, and UV transparent gel trays are available, accommodating gels of up to 6 mm thick and sample sizes from 5 to 500 μl. The 6½-inch wide gel tray will easily accommodate up to 20 samples in a run. Up to three gel trays (as many as 60 samples) may be loaded in the electrophoresis tank and separated in a single run. The unit provided includes the electrophoresis tank (with platinum electrodes), a glass sample tray, and a 13-position sample well forming comb and comb support.



Atlantis Model VQS vertical slab gel electrophoresis chamber

For high resolution slab gel electrophoresis Atlantis produce the Model VQS vertical slab gel electrophoresis chamber which combines a simplified design with study construction. Easy setup is obtained through the use of silicone rubber sealing gaskets and a single action speed clamping system. This allows rapid mounting of the electrophoresis plates without the bother of sealing grease, removable spacers or pinch clamps. The unit utilises Lucite electrolyte chambers and glass electrophoresis plates which are chemically inert and compatible with most agarose and polyacrylamide gels. A 13-cm electrophoresis path provides excellent resolution. Optional bevelled-top gel plates make two-dimension electrophoresis simple to set up. Electrical connectors are standard banana plugs and the standard apparatus includes one set of glass gel plates with integral 13-place well forming comb, 1.5 mm thick spacers, a plate centering aid and all necessary silicone rubber gaskets.

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A Chandos power supply for electrophoresis

CHANDOS INTERCONTINENTAL

Chandos Intercontinental manufacture a wide range of electrophoresis power units which are suitable for use throughout the world. This follows developments which enable a variation of the standard current/voltage stabilisation units to be used also for constant power (wattage). The output of the models available varies from 500 V at 100 mA to 10,000 V at 250 mA, the most popular being 2,500 V at 250 mA. Non-standard models are readily available. Complimentary to production of power supplies Chandos offer a range of horizontal and vertical baths for paper and cellulose acetate — and also a cooled tank for gel or slab electrophoresis. A complete unit incorporating refrigeration is available for high voltage paper electrophoresis and preparative techniques.

For radioimmunoassay Chandos produce the Mini-Assay — a low-cost counting unit primarily designed for radioimmunoassay and which has wide applications in medical research and teaching. The Mini-Assay Type 6-20 comprises a high voltage supply, discriminator and scaler with crystal controlled timer in single unit. Integrated circuits are used in conjunction with other modern techniques and components to make a compact and reliable instrument. The instrument itself is designed to work in conjunction with the 5-43 well counter but it will also operate most types of scintillation detectors with a single cable entry for the high voltage supply and signal. A G-M tube may also be used.

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BDH

Tris (hydroxymethyl) methylamine dodecyl sulphate and 4-picoline dodecyl sulphate are available, as high purity materials, from BDH Chemicals as part of the comprehensive range of reagents for electrophoresis. A new catalogue, 'Electrophoresis Materials', is now available. The penultimate section of this catalogue lists dyes and stains. It divides the materials under their various applications, for example, protein stains, tracker dyes and RNA stains, and provides literature references for each. Kenacid blue R and PAGE blue 83 are replacements for Coomassie blue R-250, and PAGE blue G-90 replaces Coomassie blue G-250 from BDH.

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TAGO INC.

The use of antisera capable of detecting polyvalent human immunoglobulin levels in sera provides a rapid and reliable means of screening patients for multiple myeloma or other immunological abnormalities. Tago offers goat antisera to polyvalent human immunoglobulins, which recognise not only IgG, IgA and IgM, but also detect the presence of IgD and IgE in combination with either kappa or lambda light chains, to provide broad spectrum screening capability. These polyvalent antisera are synthetic mixtures of antibodies of diverse specification — anti-IgA, anti-IgM, anti-kappa and anti-lambda are mixed to make these polyvalent preparations. Tago offers a choice of polyvalent antisera produced from the whole goat serum and from the immunoglobulin fraction of the goat serum.

Tago antisera to human immunoglobulins

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MSE

The Elphormat 3, available from MSE, is a clinical densitometer featuring automatic scanning of up to 12 electrophoresis separations. Scan length is selected digitally and the results are automatically printed out in mg per cent for each protein peak. Results are printed actually on the scan and for those scans containing artefacts or where separations are not complete, the integration points may be marked on the scan by hand, before quantitating. The Elphormat is particularly fast since it produces a complete scan with results in less than 20 s. Sample holders are available to suit most diagnostic systems and these may be specified by the pending purchaser. The sizes of the peaks are related to the size of the albumin peak, for serum protein separations, in order to aid visual interpretation. The Elphormat 3 is the ideal instrument for the clinical laboratory, with a sizeable electrophoresis workload, which requires quantitation.

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E-C CORPORATION

The E-C Corporation manufacture a range of electrophoresis items which may be obtained from the UK agents, Camlab Ltd of Cambridge. Of particular interest are the E-C vertical gel electrophoresis system and the EC910 transmission densitometer.

The vertical gel system offers a flexible and versatile gel media system and a complete simple-to-use apparatus for analytical or preparative work. Any gel media including agar, agarose, silica, starch, polyacrylamide and composites of polyacrylamide and agarose can be used. Polyacrylamide gel is recommended and gives superior differentiation because it has the unique capability of predictable pore size regulation to achieve molecular sieving. The E-C vertical cell consists of only two Plexiglass sections which are joined by clamps. This makes it easy to set up and operate. Separation time depends entirely on the complexity of the protein sample.

The EC910 transmission densitometer is a low-cost, compact, and easy-to-operate instrument. It will accept all transparent supporting media and has interchangeable filters in the visible range. The densitometer can be coupled to any potentiometric recorder, but if the recorder does not have integrating capabilities subsequent quantitation of the scan trace can be made using the instrument's built-in electronic integrator. The versatility offered by the EC910 permits the comparison advantages of gel slabs to be maintained throughout quantitation, yet the slit size of 0.3 × 3 mm will scan the geometrical centre of standard 5 mm diameter gel columns.

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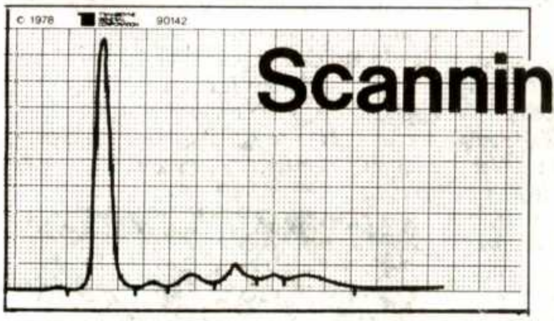

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BOEHRINGER MANNHEIM

The Densitometer 3110 made by Labora Mannheim is available now in the Uk from BCL of Lewes. This inexpensive but precise instrument scans, computes and evaluates proteins, lipids and isoenzymes on cellulose acetate and gel strips. The Densitometer 3110 is easy and quick to use. Within 60 s the Densitometer scans the total surface of a normal pherogram, draws the curves and separates the individual fractions by setting minimas. The scanning range is infinitely variable between 17 and 60 mm and is marked by two light spots. A choice of filters and slit width enables the instrument to scan macro, semi-micro and micro separations on common media which have been stained either blue or red. If the minima set by the instrument are found unsuitable then a second run using manual mode may be performed. As the scan proceeds depressing of the 'man' button sets new minima and the value of the fraction is recorded. The Densitometer 3110 is precise. Zeroing is automatic and as the values of the pherogram are intensified according to the stain, an optimal curve is always drawn. This is essential for correct observation when dealing with different pherograms. Reproducibility of 0.5 can be obtained. As the scan proceeds the instrument simultaneously stores up to 9 individual values which can be recalled at the touch of a button. Values are displayed in the order that the fractions are detected.

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M.I. SCIENTIFIC

M.I. Scientific are the United Kingdom representatives for the Helena Laboratories range of scanning densitameters and electrophoresis diagnostic kits. Helena offer a wide range of scanning integrating and computing densitometers for routine and research applications. All systems are designed to scan and integrate a variety of separated patterns on all commonly used membranes including cellulose acetate, thin layer plates, polyacrylamide gel and starch gel. The systems vary only according to their degree of automation, sample accommodation and wavelength range. The list of proven electrophoresis methodologies available from Helena includes the separation and quantification of serum proteins, lipoproteins, alkaline phosphatase isoenzymes (specific liver/intestine test), CPK and LDH isoenzymes, haemoglobins, HDL cholesterol, HbF, simple methods for haemoglobin A_2 and haemoglobin A_{1c} determination (accurate to 0.2%) plus immunoelectrophoresis and immunodiffusion methods.

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NEW ENGLAND NUCLEAR

NEN has introduced another in its family of RIANEN assay systems. The ferritin [^{125}I] radioimmunoassay kit is designed to measure ferritin levels in serum or plasma. The kit provides a sensitive and accurate assay system over a wide range of values. This system utilises a solid phase second antibody separation, a feature which reduces the usual second antibody incubation time and results in a firm, highly visible pellet. The kit provides all necessary reagents colour-coded for convenience.

The NEN system for radioimmunoassay of ferritin

Also new from NEN is the EN^{3}HANCE autoradiography enhancer. This product has been designed to greatly reduce the time necessary for researchers to visualise gels by fluorography, at the same time providing improved resolution. Compared to traditional methods, gel preparation is faster, because the procedure has eliminated several steps. The product contains no dimethylsulphoxide, and possesses an unusually high flash point (118°F, 48°C), for improved safety. Rapid visualisation and better detail are provided for carbon-14 and sulphur-35 labelled gels, as well as for lowest level tritium labelled material. EN^{3}HANCE may be used with equal ease for polyacrylamide, agarose, and mixed gels, including those stained with standard laboratory stains and dyes. EN^{3}HANCE technical bulletin available on request.

Circle No. 94 on Reader Enquiry Card.

VITATRON

The Vitatron MPS densitometer system is an analysis unit, performing determinations by densitometry, fluorescence absorbance and nephelometry. Scan speed and wavelength selection is variable as is resolution, down to 100 microns. The scan is relayed to a Vitatron 2001 chart recorder where the information can be quantified, or retransmitted to an external integrated system. The MPS densitometer is capable of scanning all types of electrophoretic media, including uncleared cellulose acetate strips. Vitatron MPS is the ideal analytical instrument for those laboratories which may only have a small scanning requirement and who would want their instrumentation to be as flexible in its application as possible.

The Vitatron thinlayer densitometer (TLD) is a purpose-built densitometer intended for the exact quantitation of information from electrophoresis strips and TLC plates. It has the capability of scanning in the transmission, reflectance, fluorescence and fluorescence quenching modes, and will accommodate slab gels up to a size of 20 cm × 20 cm. Wavelength and scan speed are selected by the operator, as is the resolution, down to 100 μm. Information is relayed in an analogue fashion to a chart recorder, which has the facility for retransmitting the output to integrators or other data handling systems. The TLD by virtue of its flying spot analytical system is the instrument of choice for scanning with the highest possible accuracy and precision.

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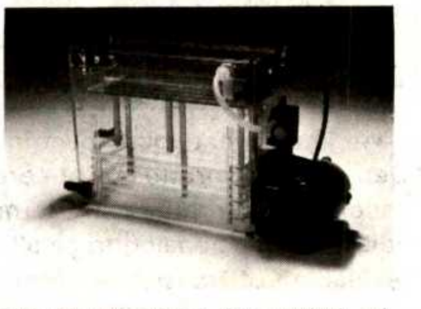
PHARMACIA

The Pharmacia gel electrophoresis apparatus GE-2/4 is the central component of an integrated PAA vertical gel electrohoresis system. The unit is designed for a wide variety of applications including SDS-PAGE, gradient gel elecrophoresis, two-dimensional work and isolectric focusing in both rods and gel slabs of various dimensions. The GE-2/4 will handle 1–4 slabs 80 mm wide and 80 or 140 mm long, 1 or 2 slabs 180 mm wide and 140 mm long, or up to 20 gel rods up to 140 mm in length. Gel slab thickness is 2.7 mm but a 0.7 mm size is available for autoradiographic applications. The unit is moulded in transparent TPX, a tough plastic resistant to all common buffers. The gaskets that locate the gels have been redesigned and are now made in a soft silicone rubber that ensures a leak proof seal and easy cassette fitting. Buffer recirculation combined with an efficient cooling system mean that high power inputs (200 W) can be used to give fast separations and reliable results. The buffer compartments can be isolated by turning a valve when separate buffers are required for IEF work. The GE-2/4 apparatus forms part of the wide range of systems for gel electrophoresis availble for Pharmacia.



The GE-2/4 system for vertical gel electrophoresis from Pharmacia of Uppsala

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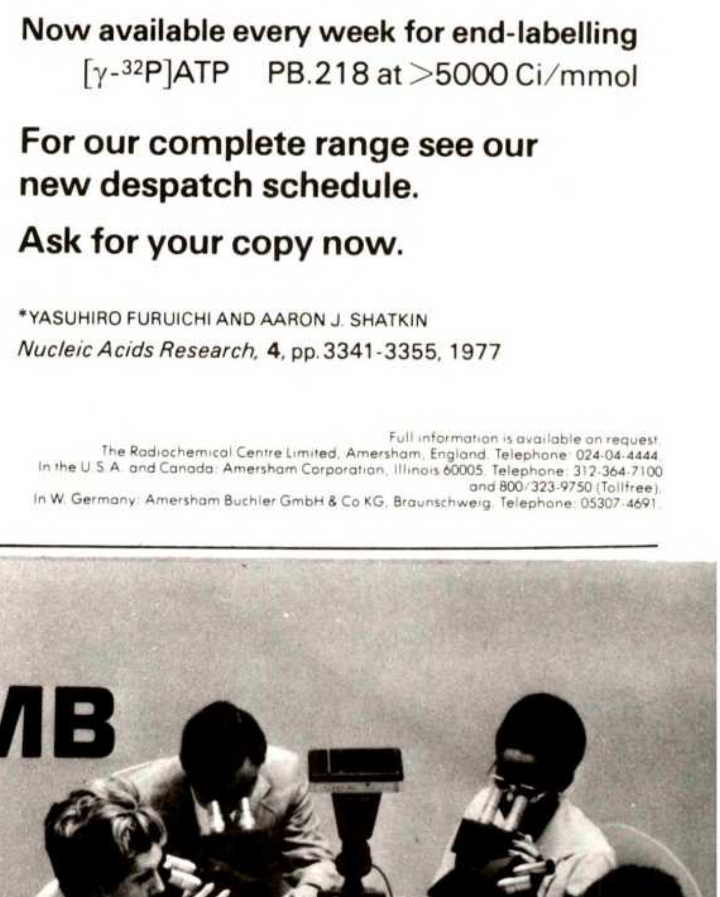

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CLINICAL ASSAYS/TRAVENOL

Clinical Assays Inc. introduce the new GammaDab [^{125}I] folate radioassay kit, which offers the convenience of room temperature denaturation instead of boiling, and a new step-saving separation system that requires no charcoal. This simple assay is run entirely at room temperature using a single tube. It take less than 30 min of technician's time, reducing total assay time by 20%. Other features include human serum N^5-MTHFA standards and a recovery of N^5-MTHFA typically greater thean 94% at all levels. Binder and separator are combined into one easy-to-pipette reagent consisting of a milk folate binder bound to a pre-precipitated antigen-antibody matrix. Once tracer and sample have competed for sites on the binder, the matrix provides a means of separation of bound from free folate. Assay tubes can be immediately centrifuged and bound tracer counted in the pellet. The kit will assay folate levels in either serum or whole blood. Also from Clinical Assays is the Gamma-Coat [^{125}I] free/total T_4 RIT kit which provides a rapid method of obtaining a free T_4 value. Detailed data sheets on the methods for both folate and T_4 assays may be obtained by circling the number indicated below on the reader enqury card.

Circle No. 97 on Reader Enquiry Card.

BULCHLER INSTRUMENTS

Buchler of Fort Lee, New Jersey has introduced a junior version of its popular 3-1500 power supply to provide a reliable, lower voltage method for electrophoresis applications. This new unit, the DPS-350 uses push-button controls to select the appropriate output ranges and regulations modes. Constant voltage and constant current levels may be selected up to 350 volts d.c. and 100 mA. This output is controlled by a 4-turn potentiometer and offers user full burn-out protection. This unit will automatically switch over from one mode to another whenever the load demand is excessive in the original mode. The large bright IED display makes for easy and precise readings from any reasonable distance within a lab. Buchler produce a full range of electrophoresis products, including a digital 1,000V power supply and analytical and preparative electrophoresis apparatus.

Circle No. 98 on Reader Enquiry Card.

NORTHEAST BIOMEDICAL

Northeast Biomedical Laboratories have published their new catalogue for listing descriptions of viral, bacterial and immunological antisera and conjugates for used in both routine and research laboratories. A new feature of the catalogue is the incorporation of three methods for evaluating the enzyme-conjugated antisera in the ELISA and EIA systems. This is supplemented by a histochemical method using amino-ethyl carbazole for peroxidase and PAP complex techniques. Each group of products is supported by an explanatory text dealing with the preparation of the immunogen. The important advantages of *unabsorbed* antisera and of antibodies to specific Fc fragments are discussed. The catalogue should prove of interest to most laboratories working on the measurement of antibody response where specific immunoglobulins are assayed as well as general laboratories involved in microbiology, haematology and histochemistry.

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BURKARD SCIENTIFIC

Burkard Scientific are agents in Southern UK for Helena and Pleuger electrophoresis equipment. The Helena range includes everything required for cellulose plate analysis, form the very comprehensive choice of plates and associated chemicals to the routine and research densitometers, which are of the highest quality. Everything the cellulose plate specialist needs is included in the catalogue. Pleuger specialise in equipment for slab gel electrophoresis and the range includes many well-tried variants.

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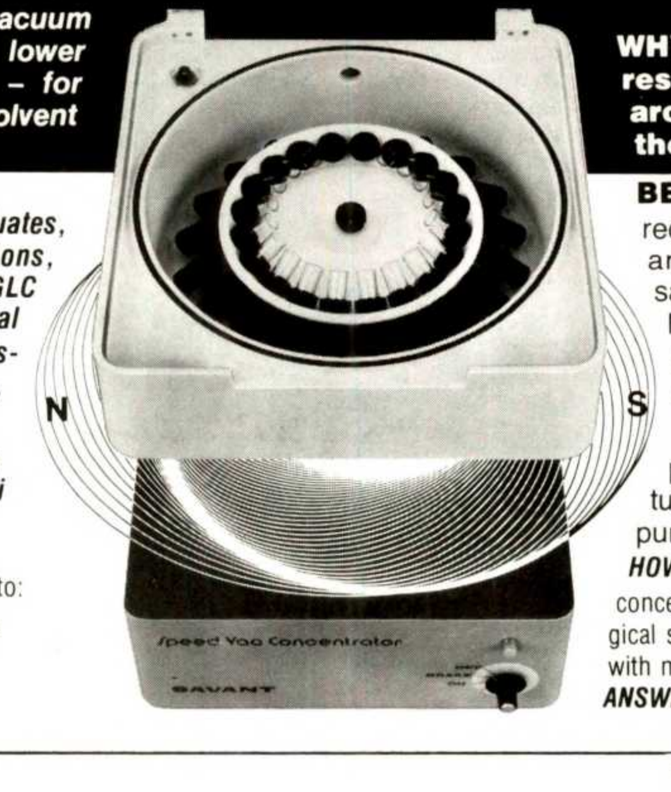

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newly on the market

Flow cytometer. Ortho Instruments is introducing a highly sensitive versatile cytometry instrument known as the ICP-22 flow cytometer. Easy to operate and maintain, the unit is capable of high resolution fluorescence measurements and allows the use of the widest ranges of dyes available. Its mercury arc lamp gives excitation at wavelengths used by fluorescence microscope users. Cells are presented to the ICP flow cytometer in liquid suspension and transported to the measuring point in the flow cell by suction. A self-contained vacuum pump supplies the suction which reduces the pressure in the waste container at the system's output. The optics of the ICP Flow Cytometer are identical to those of a fluorescence microscope.
Circle No. 71 on Reader Enquiry Card.

Flame-resistant laboratory coats. Gallenkamp. New flame-resistant laboratory coats are now available from Gallenkamp. The coats have a Proban flame retardant finish which meets the requirements of BS 3120 (3119). Proban-treated fabric will resist both the passage of heat and the spread of flames without any smouldering or afterglow. The flame resistance of the coats is not reduced by normal methods of washing or by dry cleaning. Made of cotton the coats are free from static build-up. The coats for both men and women are front fastening with sewn on buttons and two patch pockets.
Circle No. 72 on Reader Enquiry Card.

Marker protein for density gradient centrifugation. Pierce announces the introduction of BSA-Black, an internal standard protein useful for sedimentation coefficient calculations. BSA-Black (amido black 10 β-stained bovine serum albumin) conveniently replaces the use of ^{14}C-BSA to detect BSA in a mixture of other proteins. It does not interfere with the detection of isotopically labelled fractions in the sample and can be easily monitored at 625 nm. BSA-Black, product no. 26640, is supplied in 20-mg packages.
Circle No. 73 on Reader Enquiry Card.

Stain removers. Cambridge Chemical Products. Camco Erado-Sol (liquid) and Camco Erada-Stain (cream) are effective biological stain removers which rapidly remove most stains from hands, glassware and laboratory equipment. They are easy to apply; simply rub in and the stain is gone in a matter of seconds. Rinse with water. Non-irritating to the skin.
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Variable speed micro-shaker. Dynatech have developed an improved microshaker specially designed for shaking all types of microtitre plate. Designated the AM169 Varishaker, the new machine ensures optimum agitation for the re-suspension of cell aggregates and for the mixing of reagents and sera. On the AM169, speed can be reduced or increased as required via a single control, and can be run with up to 4 microtitre plates in position; the plates can be loaded or unloaded without switching off. The vibrational mode is such that all wells receive the same degree of agitation.
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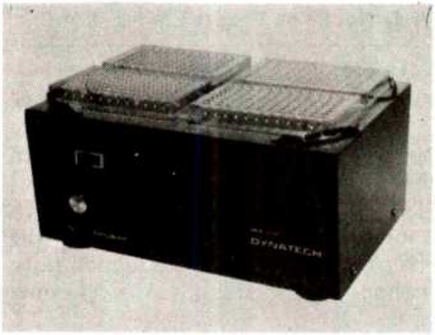

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Mobile table. Bigneat. Labplan design from Bigneat was the result of a very precise programme of functional research governed by usage. The materials used have been selected for their strength, durability, resistance to acids and solvents. Features of Labplan are the 20-l spillage tray, four double-locking castors, a service channel which runs below the spillage tray to accommodate cables and pipes, and a large drawer for the storing glassware or samples. The open storage shelves can be used for apparatus, thus keeping the work area clear.
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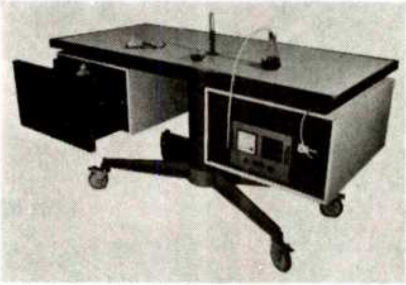

The Labplan mobile table from Bigneat

These notes are based on information provided by the manufacturers. For further details circle the appropriate numbers on the Reader Enquiry Card bound inside the cover.

Densitometer. Kratos. The SD 3000 spectrodensitometer for the quantitative analysis of thin layer chromatograms is a sophisticated thin layer chromatogram scanner capable of making absorbance and fluorescence measurements of spotted thin layer separations on TLC plates, paper or film, as well as agarose gels, disc gels, slab gels and electrophoretic media. Because of the precision of the SD 3000 and the resolution of the incident light beams, it is equally suited for the analysis of HPTLC plates. The SD 3000 may be operated in either the single beam or double beam ratio mode. The SD 3000 permits the user to choose from an array of compatible accessories according to application and budget.
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Materials for radioactive iodination of proteins. Pierce. Iodo-Gen (1,3,4,6-tetrachloro-3α, 6α-diphenylglycoluril, product no. 28600) from Pierce is extremely stable and virtually insoluble in water, allowing rapid iodination in the solid phase with aqueous solutions of I^- and proteins. The incidence of side reactions with this method is negligible. The solubility of Iodo-Gen in chloroform permits 'plating' aliquots of any amount on the walls of the iodination vessel, which can be stored indefinitely before use. Iodinations are terminated by simply decanting the reaction solution from the solid Iodo-Gen. Specific radioactivities of 1×10^5 c.p.m. of ^{125}I per mg of protein and efficiences of 60% are easily achieved. Methyl-*p*-hydroxylbenzimidate · HCl (MPHBIM) product no. 26048) is a water soluble, readily iodinated imidoester. It is moderately stable at *p*H 9.5 and 37°C in aqueous solutions. The amidine linkages formed with proteins are specific for ε-amino groups of lysyl residues and do not alter the net charge of the native protein.
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Field/laboratory nephelometer. The Turner Designs Model-40 field/laboratory nephelometer with a flow-through cell attachment is now available. Using a suitable inverter, the Model-40 can be operated from a battery or car cigar lighter socket, to provide reliable turbidity measurements of raw or finished water in less than 5 min from setting-up. Alternative operation as a laboratory or on-line instrument is also possible. The cell has a volume of less than 30 ml. An optional 4–20 mA signal transmitter also permits installation up to 5 miles from a control panel or central office.
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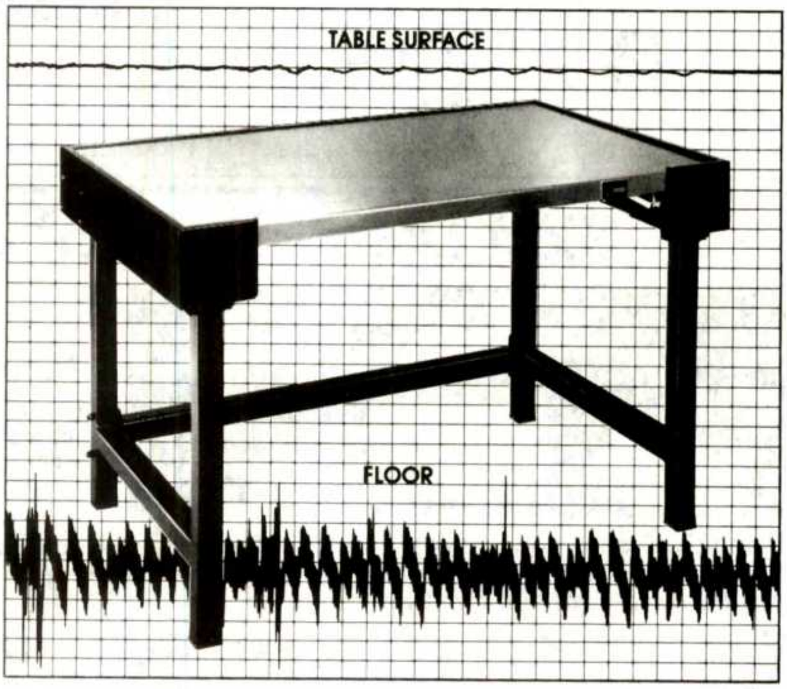

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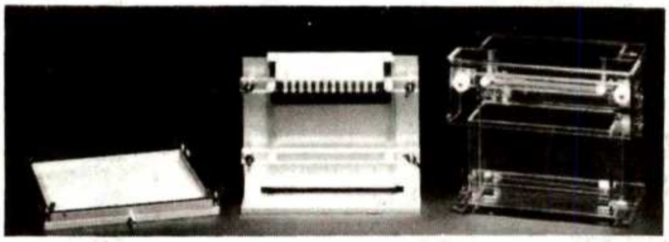

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announcements

Awards

The following awards of medals have been made by the Council of the Royal Society. **The Copley Medal to Dr M.F. Perutz,** in recognition of his distinguished contributions to molecular biology; **The Davy Medal** to **Professor J. Chatt,** in recognition of his distinguished contributions to transition metal chemistry and the understanding of catalysis; **The Sylvester Medal** to **Professor G. Higman,** in recognition of his distinguished and profoundly influential contributions in algebra; **The Hughes Medal** to **Professor R.J.P. Williams,** in recognition of his distinguished studies of the atomic structure of proteins in solution using NMR.

The Royal Society Mullard Award for 1979 is to be presented to **E.M. Eltis** and **G.L. Wilde** in recognition of their contribution to the design and development of the Rolls-Royce RB211 turbofan engine. The award is made annually by the Council of the Royal Society in recognition of an outstanding contribution to the advancement of science, engineering or technology which has led directly to national prosperity in the UK.

The Council of the Royal Society has awarded the following three Royal Medals for 1979: to **Sir Charles Frank,** in recognition of his outstanding original contributions to the theory of crystal growth, dislocations, phase transformations and polymers; to **Professor H.W. Kosterlitz** in recognition of his distinguished work on narcotics leading to the discovery of the enkephalins; to **Dr V.E. Cosslett,** in recognition of his outstanding contributions to the design and development of the X-ray microscope and the scanning electron microprobe analyser (scanning electron microscope).

Professor Hermann Lehmann has been awarded The Wellcome Prize for 1979. The Prize, which is in the gift of the Association of Clinical Biochemists, is awarded annually for outstanding scientific contributions to the quality of Clinical Biochemistry as practised in the UK.

The L.R. Wager Prize, for outstanding contributions to the study of volcanic rocks has been awarded to **Dr D.H. Eggler** of the Department of Geosciences, Pennsylvania State University.

The Trustees of the Lady Tata Memorial Trust for research in leukaemia have made the following international awards for the academic year 1979–80. **K. Koubek** (Czechoslovakia); **S. Venuta** (Italy); **B. Azzarone** (Italy); **H. Bhayani** (UK); **E. Harlow** (US); **H. Jacobs** (UK).

The Paul-Martini-Prize 1979 (DM20,000) has been awarded to two German scientific teams. The prize which is intended to promote international cooperation in the field of clinical pharmacology, has been issued since 1969 by the Medizinisch Pharmazeutische Studiengesellschaft e. V. (Frankfurt) in cooperation with the Deutsche Gesellschaft für Medizinische Dokumentation, Informatik und Statistik e. V. (Köln). **Professor Dr. Klaus Breddin,** Head of the Division of Angiology at the Centre of Medicine of the J.W. Goethe University (Frankfurt) and his group receive the prize for a multicentre epidemiological study. **Dr Hans-Jörg Ruoff** of the Department of Pharmacology, University of Tübingen, and his group receive the prize in recognition of a pathophysiological study of the human gastric mucosa and the regulation of gastric secretion by H_1 and H_2 receptor blocking drugs.

The American Institute of Biological Sciences (AIBS) has given its highest honour — the AIBS Distinguished Service Award to **Dr Lee Talbot,** Swiss-based Director of Conservation and Special Scientific Advisor to the World Wildlife Fund.

The recipients of the Wolf prizes for 1979 are: for Agriculture shared by **Professor Jay L. Lush** (Iowa State University) **Dr Sir Kenneth Blaxter** (The Rowett Research Institute); for Mathematics shared by **Professor Emeritus Jean Leray** (College de France, Paris) and **Professor Emeritus André Weil** (Institute for Advanced Study, Princeton); for Chemistry by **Professor Emeritus Herman F. Mark** (Polytechnic Institute of New York); for Physics shared by **Professor George Uhlenbeck** (Rockefeller University) and **Professor Giuseppe Occhialini** (University of Milan); for Medicine shared by **Dr Roger W. Sperry** (California Institute of Technology) **Dr Arvid Carlsson** (Gothenburg University) and **Dr Oleh Hornykiewicz** (University of Vienna).

A memorial service for the late Professor Sir Ernst Chain FRS will be held at the Central Synagogue, Great Portland Street, London W1 on Sunday 21 October at 6.15 pm.

The Meldola Medal for 1978 has been made to **Dr John Evans** for his work on metal cluster compounds. The next award will be made in 1980 to the chemist who, being a British subject and under 30 years of age at 31 December 1979, shows the most promise as indicated by his or her published chemical work brought to the notice of the Council of the Royal Institute of Chemistry, 30 Russell Square, London WC1, UK before 31 December, 1979.

The biennial award in Carbohydrate Chemistry (sponsored by Tate & Lyle Ltd) consisting of an inscribed document and monetary prize of £100 will be made to the Fellow of the Chemical Society who shall have published during the year in question, and in the immediately preceding five years, the most meritorious contributions to the knowledge of any aspect of carbohydrate chemistry, and who, at the date of publication, were under the age of 36 years. The recipient must be a citizen of the UK, and the major part of the relevant scientific contributions must have been carried out and published in the UK. Applications (by 31 October 1979) to S.S. Langer, The Chemical Society, Burlington House, London W1, UK.

Three Corday-Morgan medals and prizes for work in different branches of Chemistry each consisting of a silver medal and monetary award of 250 guineas will be made in respect of the year 1978. These will be made to the chemists of British nationality have published during the year in question and in the immediately preceding five years, the most meritorious contributions to experimental chemistry in their respective fields. Candidates should be under the age of 37 years at 31 December 1978. Applications or recommendations in respect of the year 1978 must be received not later than 31 December 1979 to The Chemical Society, Burlington House, London W1, UK.

Fellows of the Chemical Society should note that the annual award of the Marlow Medal and grant of £100 is based on publications (not necessarily in the Transactions) on any subject normally published in JCS Faraday Transactions I and II. Applicants should be under the age of 33 years on 1 January 1980, and applications may be made by either the candidate or on his behalf by another Fellow of the Society. Application by 1 January 1980 to Mrs Y.A. Fish, Faraday Division, The Chemical Society, Burlington House, London W1, UK.

The Beilby Awards are made to British investigators in recognition of independent, exceptional and original work carried out over a period of years and involving the development and application of scientific principles in any field related to the special interests of Sir George Beilby, (chemical engineering, fuel technology or metallurgy) in their modern interpretations. The Awards are intended to encourage younger men and women (normally under the age of 40). Such work may be brought to the notice of The Convenor of the Administrators, Sir George Beilby Memorial Fund, The Royal Institute of Chemistry, 30 Russell Square, London WC1, UK.

Nominations are invited from Palynological societies and institutions for the award of the Erdtman Medal for the years 1977 and 1979. The chief aim of the award is to accord recognition to outstanding contribution to palynology in its widest sense, embracing botanical, geological, medical, agricultural and other possible aspects. The nominations should reach the Secretary, Prof G. Erdtman International Medal for Palynology Award Committee, National Botanical Research Institute, Lucknow-226001, India by 31 October 1979.

The Naples Zoological Station has arrangements with the Royal Society for British marine biologists to work there on topics of their own choice. Applications for table-time may be made through the Royal Society, the Physiological Society or one of the 10 contributing UK universities (details from the Executive Secretary of the Royal Society, 6 Carlton House Terrace, London SW1, UK). The Royal Society will also consider applications for financial support through its European Science Exchange Programme or from its Browne Fund for marine research.

Up to six Wain Fellowships are available each year to enable younger research scientists to work or study abroad at an academic, industrial or agricultural institution of their choice for a period of up to three months. The Fellowships may be awarded in any science relevant to agriculture, including animal and veterinary sciences. The closing date for applications are 1 April and 1 October annually. Further particulars from the Secretary, Agricultural Research Council, 160 Great Portland Street, London W1, UK.

The Dennis Stanfield Memorial Fund has been established to assist persons of scientific merit to undertake botanical research on tropical African plants. The third award, of £150, will be made in 1980. Application forms from the Executive Secretary of the Linnean Society of London, Burlington House, Piccadilly, London W1, UK by 30 October 1979.

Applications are invited for the 1979 Karl Heinrich Gyr and Heinrich Landis Commemorative Prize. This prize (£500) is awarded annually for practical contribution to the advancement of electrical or electronic science or engineering. Applications for the award should be submitted to the Secretary, The Institution of Electrical Engineers, Savoy Place, London WC2, UK.

The Gottschalk Medal recognises distinguished research in the medical or biological sciences by younger scientists. Candidates must be under the age of 36 years (and not Fellows of the Academy) at the closing date for nominations, and their research must have been carried out mainly in Australia. Applications to The Executive Secretary, Australian Academy of Science, PO Box 783, Canberra City, Act 2601, Australia.

Learned Societies, academies, universities and individuals in industry throughout the world are now being invited to nominate candidates for the Sixth Marconi International Fellowship (a $25,000 grant). The 1980 Fellowship will be made to a person who has made an outstanding contribution to the improvement of communications in developing countries. Applications to Marconi International Fellowship, Aspen Institute of Humanistic Studies, 1919, 14th St, No. 811, Boulder, Colorado 80302.

The Pawsey Medal is normally awarded annually. It recognises outstanding research in experimental physics by younger scientists. Candidates must be under the age of 36 years at the closing date for nominations, and their research must have been carried out mainly in Australia. Applications to The Executive Secretary, Australian Academy of Science, PO Box 783, Canberra City, Act 2601, Australia.

Dr Jesse Roth is to share the Diaz-Christobal Prize with **Dr Pierre De Meyts** following publication of mapping of the residues responsible for the negative cooperativity of the receptor-binding region of insulin, in *Nature* **273**, 504 (1978).

HEART (Heart Exploration and Research in Therapy Ltd) is a new charity dedicated to supporting research into coronary artery disease that has been set up. Initially preference will be given to grant applications covering the field of non invasive diagnosis but applications for funding for work in related areas of coronary artery disease will also be considered. Applications to HEART, 47 Wimpole St., London W1, UK.

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Appointments

Dr K. D. Tocher, Coordinator, Management Sciences and Computer Technology, British Steel Corporation, has been appointed to the Chair of Operational Research at Southampton University with effect from 1 May 1980.

The Agricultural Research Council has announced the appointment of **Dr Allen J. Bailey** as Director of its Meat Research Institute at Langford near Bristol, from 1 September 1979.

J.T.M. Taylor has been elected President of the Institution of Mining and Metallurgy for the session 1980–81 in succession to Dr D.A. Temple.

Dr Lewis T. Chadderton Professor of Physics at the H.C. Ørsted Institute of the University of Copenhagen will succeed Dr A.L.G. Rees as Chief of CSIRO's Division of Chemical Physics.

Dr David Smiles has been appointed to head CSIRO's Division of Environmental Mechanics. He will succeed **Dr J.R. Philip** who has been appointed Director of the organisation's Institute of Physical Sciences from February 1980.

Dr A.G. Sykes has been appointed to the Chair of Inorganic Chemistry at the University of Newcastle upon Tyne, from 1 October 1979.

Dr Ian Graham-Bryce, from Rothamsted Experimental Station, has been appointed Director of East Malling Research Station in succession to **Dr A.F. Posnette** who retired in July.

Dr Jeffrey W. Davies of the University of Wageningen has been appointed Head of the Virus Research Department at the John Innes Institute in Norwich with effect from 1 October 1979.

Professor Sir David Phillips has been appointed Fullerian Professor of Physiology at the Royal Institution from October 1979, in succession to Professor Max Perutz.

Professor Keith Vickerman has been appointed to the newly established second Chair in Zoology and head of department in the University of Glasgow, with effect from 1 October 1979.

Lawrence Theodore Threadgold, has been appointed to the chair of Zoology at The Queen's University of Belfast.

Professor Michael Thompson Professor of Experimental Physics at the University of Sussex, has been named to succeed **Dr Frank Thistlethwaite** who will be retiring in September 1980.

Reports & Publications

UK & Ireland — July

Department of Industry. Technology and the Environment. Noise -- Reports from Overseas Posts, No.11, 1979. Part 2: Austria, France, Germany and Italy. Pp.39. Part 3: Argentina, Colombia, Egypt, Iran, Israel, Japan, Turkey and USA. pp.56. (London: Department of Industry, Abell House, John Islip Street, SW1, 1979.) (307

The Electricity Council. Annual Report, 1978-k79. Pp.36. (London: The Electricity Council, 30 Millbank, SW1, 1979.) (307

Natural Environment Research Council. Institute of Terrestrial Ecology Annual Report, 1978. Pp.115. (Cambridge: Institute of Terrestrial Ecology, 1979.) (307

Second Report from the Select Committee on Science and Technology, together with the Minutes of Evidence Taken Before the Genetic Engineering Sub-Committee and Appendices, Session 1978-79. Recombinant DNA Research --Interim Report. Pp.xiii + 267. (London: HMSO, 1979.) £6 net. (307

Freshwater Biological Association. Forty-seventh Annual Report for the year ended 31 March 1979. Pp.118. (Far Sawrey, Ambleside, Cumbria: Freshwater Biological Association, 1979.)£2. (307

A Handbook to Estimate Climatological Concentration, Deposition and Horizontal Fluxes of Pullutants on a Regional Scale. By L. Machta. (MARC Report No.11.) Pp.87. (London: Monitoring and Assessment Research Centre, Chelsea College, University of London, 1979.) £5; $10. (307

Code of Practice for the Pharmeceutical Industry, Together with the Constitution and Procedure for the Code of Practice Committee. Fifth edition. Pp.28. (London: The Association of the British Pharmaceutical Industry, 162 Regent Street, W1, 1978.) (317

Office of Population Censuses and Surveys. Cancer Statistics: Registrations. Cases of Diagnosed Cancer Registered in England and Wales, 1971. (Series MB1, No.1.) Pp.xi + 105. (London: HMSO, 1979.) £2.50 net. (317

Proceedings of the Royal Irish Academy. Section A: Mathemtical and Physical Sciences. Vol.78. No.16: On Norm Approximation of Functions of Operators in the Calkin Algebra. By I.D. Berg. Pp.143-148. £1.10. No.19: Studies of F. Centre Luminescence in CaO. By B. Henderson. Pp.181-193. £1.45. No.20: On the Critical Points of Polynomials. By Siobhán Vernon. Pp.195-198. 65p. No.22: Infrared Behaviour of Yang-Mills Theories. By C. Nash and R.L. Stuller. Pp.217-233. £1.80. No.23: Formal Models for Biological Oscillators, Derived by Using Liapunov Stability Theory in a Synthetic Mode. By H.M. Power. Pp.235-245. £1. Vol.79. No.1: The Variation on the Real Line. By R. Henstock. Pp.10. £1.30. Section: Biological, Geological and Chemical Science. Vol.78. No.17: The Karyology of *Hydrilla* (Hydrocharitaceae) from Ireland to Poland. By Romana Czapik. Pp.267-272 + plate 11. 80p.No.18: Movements of Salmon Around Ireland. XII. From the Coast of West County Cork. By D. McCarthy. Pp.273-279. 70p. No.19: Palynological Evidence for the Age of the LowerPalaeozoic Rocks of Slieve Aughty, Counties Clare and Galway. By G.T. Emo and D.G. Smith. Pp.281-292 + plate 1. £1.20. No.20: Characterisation of Glacial Deposits in South Wexford. By E.B. Culleton. Pp.293-308. £1.20. No.21: Water Quality in an Ulster Nature Reserve. By R.B. Wood and C. Carter. Pp.309-322. £1.10. No.22: Isozymes of Interspecific Hybrids of the Fish Family Cyprinidae. By T.F. Cross. Pp.323-330 + 4 plates. £1.05. No.23: Intrazeolitic Chromium Complexes. By B. Coughlan, W.A. McCann and W.M. Carroll. Pp.331-340. £1. Vol.79. No.1: The Social Hymenoptera of County Wexford. By F.J. O'Rourke. Pp.1-14 + plate 1. £1.05. No.2: The Arthropod Fauna Associated with Cattle Manure Applied as Slurry to Grassland. By J.P. Curry. Pp.15-27. £1.20. No.4: The Geomorphology of Offshore Sand Bars on the North Coast of Ireland. By R.W.G. Carter and K.J. Kitcher. Pp.43-61 + 3 plates. £1.65. (Dublin: Royal Irish Academy, 1978 and 1979. (317

— August

The Radiochemical Centre Limited. Eighth Annual Report, 31st March 1979. Pp.20. (Amersham: The Radiochemical Centre Limited, 1979.) (18

University of Cambridge: Department of Engineering. Report of the Head of the Department for the Academic Year 1977/78. Pp.26. (Cambridge: University Engineering Department, 1979. (38

The Soil Survey: England and Wales. Annual Report 1978. Pp.38. (Harpenden, Herts.: Soil Survey of England and Wales, Rothamsted Experimental Station, 1979.) (68

The Protection of Wild Birds. By R. Paul Davis. Pp.18. (Chichester: Barry Rose (Publishers), Ltd., 1979.) £1.25. (68

Central Water Planning Unit. Annual Report, 1978/79. Pp.68. (Reading: The Central Water Planning Unit, Reading Bridge House, 1979.) (68

Ministry of Agriculture, Fisheries and Food: Directorate of Fisheries Research. Technical Report No.50: The Field Assessment of Effects of Dumping Wastes at Sea. 4. A Survey of the Sewage Sludge Disposal Area Off Plymouth. By R.A. Eagle, P.A. Hardman, M.G. Norton and R.S. Nunny. Pp.24. (Lowestoft: Directorate of Fisheries Research, 1979.) (78

Health and Safety Executive. Ceramics: Health and Safety, 1971-1977. Pp.15. (London: HMSO, 1979.) 75p net. (98

Laboratory of the Government Chemist. Report of the Government Chemist 1978. Pp.170. (London: HMSO, 1979.) £4.25 net. (98

The Natural Environment Research Council. Research Grants in the Natural Environmental Sciences, NERC Research Grants Current on 1st October 1978. Pp.v + 64. (Swindon: NERC, North Star Avenue, 1979.) (98

Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol.281, No. 1384: The Statistical Theory of Turbulent Flames. By S.B. Pope. Pp.529-568. UK £2.65; Overseas £2.90. Vol.291. No. 1385: The Magnetoresistance of Polycrystalline Copper. By Sir Brian Pippard, FRS. Pp.569-601. UK £2; Overseas £2.25. B: Biological Sciences. Vol. 286, No. 1015: The Assessment of Sublethal Effects of Pollutants in the Sea. A Discussion organized by H.A. Cole, in collaboration with the Marine Pollution Subcommittee of the British National Committee on Oceanic Research. Pp.397-636 + 1 plates. (London: The Royal Society, 1979.) UK £16.90; Overseas £17.30. (98

Annual Report for 1978 of the Medicines Commission. Pp.120. (London: HMSO, 1979.) £3 net. (108

Office of Population Censuses and Surveys. Local Authority Vital Statistics: Vital Statistics for Administrative and Health Areas of England and Wales, 1977. (Series VS No.4.) Pp.iii + 29. £1 net. Royal College of General Practitioners/Office of Population Censuses and Surveys/Department of Health and Social Security. Morbidity Statistics from General Practice, 197172: Second National Study. (Studies on Medical and Population Subjects, No.36.) Pp.x + 197. £7 net. (London: HMSO, 1979.) (148

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United States Department of the Interior: Geological Survey. Professional Paper 1043: Subsurface Stratigraphy and Geochemistry of Late Quaternary Evaporites, Searles Lake, California. By George I. Smith. Pp.v + 130 + 2 plates. (Washington, DC.: US Government Printing Office, 1979.) (28

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APPOINTMENTS VACANT

ANALYST TECHNICIAN (Grade 4) required for Department of Geology, University of Reading. Applicants experienced in wet chemical methods for silicate analysis preferred but those with appropriate experience in general inorganic analysis by atomic absorption, colorimetric and other techniques also considered. Salary according to qualifications and experience in scale £3,018 to £3,657 p.a. (under review). Further details quoting Ref. TN69A, from Assistant Bursar (Personnel), University of Reading, Whiteknights, Reading, RG6 2AH, to whom applications should be sent as soon as possible.

3501(A)

POSTDOCTORAL POSITIONS

Opportunities for experimentalists with background in atomic physics, solid state or materials science, to study interactions of matter with radiation (electrons, photons including synchrotron radiation). Experience in one of these fields desirable. One- to two-year appointments. DM 24,000 per year tax free.

Send application, with references, to: Fritz-Haber-Institut der Max-Planck-Gesellschaft zu Hd. v. Fr. L. Lambert Faradayweg 4-6, D-1000 Berlin 33.

W440(A)

DIRECTOR

Peabody Museum of Natural History

Yale University invites nominations and applications for the position of Director, Peabody Museum of Natural History. Applicants should be recognized scholars qualified to hold professorial rank in one of the three areas of museum activity, Anthropology, Biology and Geology. Demonstrated administrative ability and familiarity with the functions and problems of a research-oriented natural history museum serving both the university and the public are desired.

The Peabody Museum, founded in 1863, serves as an interdisciplinary research and teaching laboratory, as a major repository of scientific collections, and as a university contribution to the life of the community, the latter function including public exhibits, programs and school services and other community-oriented activities.

The Director presides over a board of curators, all of whom are full-time Yale faculty, and various administrative and operational support services. An Assistant to the Director and Business Manager aid the Director in museum administration. Museum support is derived both from the university budget and, to an increasing extent, outside funding. The development of new research programs, centered in the museum, will be important tasks for the Director.

Yale University is interested in increasing the number of qualified women and minority group members in its faculty and welcomes applications from women and minority group members.

Nominations, applications and supporting material should be sent, before November 23rd, 1979, to:

Robert B. Gordon, Chairman, Directors Search Committee Department of Geology and Geophysics

Yale University
Box 2161 Yale Station
New Haven, Conn 06520

Yale University is an Affirmative Action Employer. All interested persons are encouraged to apply.

IMPERIAL COLLEGE UNIVERSITY OF LONDON

DEPARTMENT OF PHYSICS

LECTURESHIP IN PHYSICS

BLACKETT LABORATORY

Applications are invited for a number of lectureships in physics from candidates whose research interests lie in the fields of astronomy, optics and laser physics, solid state physics, spectroscopy and theoretical physics.

The successful candidates will be attached to the appropriate research section within the Physics Department. Salary on the scale £4,333 to £8,992 (under review) with superannuation plus London allowance of £740 p.a.

Applications with curriculum vitae, list of publications and the names of two referees should be addressed to Professor D.J. Bradley, Department of Physics, Imperial College, London SW7 before 8th November 1979.

Applications are invited for a

POST-DOCTORAL RESEARCH ASSISTANTSHIP

OPTICS SECTION

The successful candidate will engage in a theoretical research programme in the field of laser dynamics and non-linear optics. Applicants should hold a Phd degree (or equivalent) or be in the process of completing their doctoral thesis. A reasonable background in theoretical physics is a prerequisite and previous experience in quantum electronics would be an advantage. The appointment will be for two years and the salary will be at an appropriate point on a scale starting at £4,333 (under review) plus £740 London allowance and superannuation.

Applications, accompanied by at least two references, should be sent to Dr G H C New, Optics Section, Department of Physics, Imperial College, London SW7 2BZ.

3522(A)

UNIVERSITY OF ASTON IN BIRMINGHAM

DEPARTMENT OF BIOLOGICAL SCIENCES

RESEARCH FELLOW

Applications are invited for the above post from suitably qualified comparative physiologists to work either in the area of experimental diabetes and carbohydrate metabolism or in some aspect of fish physiology.

Informal enquiries may be made to Professor A J Matty, Head of Department of Biological Sciences (Tel Ext 6083). Commencing salary will be within the range £4,333 to £5,488 per annum on a scale rising by annual increments to £7,521 per annum.

Application forms and further particulars may be obtained from the Staff Officer (quoting Ref No 379/6) University of Aston in Birmingham, Gosta Green, Birmingham B4 7ET (tel 021 359 3611 Ext 201) to whom applications should be forwarded not later than 9th November, 1979.

3505(A)

UNIVERSITY OF BRISTOL

LONG ASHTON RESEARCH STATION

MATHEMATICAL MODELLER

Required to participate in the developmen of a model to simulate the growth and yiel of winter wheat.

The appointee will be located at Lon Ashton Research Station, will join inter institute working parties concerned wit the structure and mechanisms of th model, and will be responsible for it algebraic formulation and for some dat collation and analysis to obtain necessar parameter values.

Will also be expected to visit or stay at othe institutes on occasion.

The appointment is for a 3 year period.

Starting salary according to qualification and experience. Scientific Officer (£3,28 to £5,085) or Higher Scientific Office (£4,727 to £6,211). Non-contributor superannuation scheme.

Minimum Qualifications 2(1) Honou degree in Physics, Biophysics or Applie Mathematics, with a strong interest in pla biology. At least two years releva experience for H.S.O. grade.

Further particulars an application for from the Secretary, Long Ashton Researc Station, Long Ashton, Bristol, BS18 9A to whom applications should be sent by 8 November, 1979.

3513(A)

CERRO TOLOLO INTER-AMERICAN OBSERVATOR

EXPERIMENTAL PHYSICISTS OR ASTRONOMERS

The observatory (operated by th Association of Universities for Research i Astronomy, Inc. in Tucson, Arizon under contract with the U.S. Nation Science Foundation) has staff positio available for experimental physicists astronomers. The ideal candidates w have strong backgrounds in detect technology, low noise solid sta electronics, cryogenic techniques an preferably advanced degrees and j related experience.

The selected candidates will ha responsibility for the design an development of detector systems an associated hardware for the observatory astronomical infrared research programs

Salaries and position levels negotiab according to qualifications.

The principal work location is in t coastal city of La Serena, Chile. Liber fringe benefits, including overse allowance, observatory provid reasonable rent housing close to work, well as opportunities to participate in t observatory's research programs.

Candidate selections will be made January 31 and June 30, 1980. Se Resume to: Personnel Office, 950 Cherry Ave., P.O. Box 26732, Tucson, A 85726

W447(A)

Editor of Nature

Applications are invited from those wishing their names to be considered for this appointment, which will become vacant at the end of this year.

A brief account of past career, qualifications and experience considered relevant should be sent in writing to:

nature

The Publishing Director,
Macmillan Journals Limited,
4 Little Essex Street,
LONDON WC2R 3LF

3504(A)

THE UNIVERSITY OF NEWCASTLE UPON TYNE

EPARTMENT OF OCCUPATIONAL HEALTH AND HYGIENE

pplications are invited from men and omen for the post of:

TEMPORARY LABORATORY TECHNICIAN (RESEARCH)

o work in the Research Unit of the above epartment. The project involves the vestigation into the variation in normal ng function. Candidates should have an NC, OND or relevant City and Guilds ertificate in Physiology or Applied iology and have had three years' perience including training period. The st is tenable until 30th June, 1981. Grade salary scale £3,122 to £3,553 per annum.

Written applications, giving details of e, qualifications, experience, present lary and the names and addresses of two ferees to Dr J.E. Cotes, Department of ccupational Health and Hygiene, 21 laremont Place, Newcastle upon Tyne, E1 7RU. 3492(A)

WITHINGTON HOSPITAL

Nell Lane,
Manchester M20 8LR

SCIENTIFIC OFFICER

r National (UK) Reference Laboratory r Anticoagulant Reagents and Control.

Graduate with good honours degree in ochemistry or allied sciences required. lary £4,035 rising to £4,935.

Work will be concerned with the actical application of techniques in the oduction, research and development of boratory standards.

Enquiries to the Director of the National K) Reference Laboratory for nticoagulant Reagents and Control.

Y3481(A)

OXFORD UNIVERSITY

Applications are invited for the post of Medical Laboratory

SCIENTIFIC OFFICER

to work in the Nuffield Dept of Clinical Medicine/MRC Molecular Haematology Unit on the control of haemoglobin synthesis. The work involves studies on globin chain synthesis in thalassaemia and other inherited disorders in man as well as experimental work in sheep. Applicants should hold either a degree in biochemistry or related subjects or HNC in medical laboratory sciences. Salary on the Whitley Council Scale, £3,768 to £5,391 (£4,125 to £5,817 wef 1.1.80) depending on age and experience.

Full job description and application forms from: The Departmental Administrator, Nuffield Dept. of Clinical Medicine, Room 5801, John Radcliffe Hospital, Headington, Oxford. Closing date for applications is 1.11.79. 3495(A)

UNIVERSITY OF OXFORD

DEPARTMENT OF BIOCHEMISTRY

DEPARTMENTAL DEMONSTRATORSHIP

It is proposed to appoint a Departmental Demonstrator as soon as possible. The salary will be on the scale £3,775 to £5,008 p.a., according to qualifications and experience and with membership of U.S.S. The appointment is for a period not exceeding 3 years. The successful candidate will be expected to take part in departmental teaching and to conduct his or her own research.

Applications (3 copies) including the names of two referees, should be sent not later than 30th November, 1979 to:

The Administrator, Department of Biochemistry, South Parks Road, Oxford OX1 3QU. 3532(A)

GLASSHOUSE CROPS RESEARCH INSTITUTE

has vacancy in

PLANT PHYSIOLOGY DEPARTMENT

for scientist to initiate research programme on plant membranes and their properties.

Appointment in

HIGHER SCIENTIFIC OFFICER/SENIOR SCIENTIFIC OFFICER

grade depending on qualifications and experience. Salary scales £4,727 to £6,211 (HSO) or £5,876 to £7,864 (SSO). Applicants should have First or Upper Second class Honours degree in appropriate subject and two years experience in study of biological membranes, or equivalent experience. Non-contributory superannuation scheme.

Further details and application forms on request. Applications to Secretary, Glasshouse Crops Research Institute, Worthing Road, Rustington, Littlehampton, West Sussex, BN16 3PU, by 7 November 1979. 3497(A)

POST DOCTORAL POSITION — MEMBRANE BIOLOGY

Available January 1980, to investigate the membrane proteins of red cells infected with malaria and malarial parasites. Preference will be given to candidates with previous experience in membrane isolation and characterization of membrane proteins using biochemical, immunologic and/or electron microscopic techniques.

Send three letters of recommendation, curriculum vitae, and a statement of research interests to: Dr Irwin W Sherman, Department of Biology, University of California, Riverside, Calif. 92521. An Equal Opportunity/Affirmative Action Employer. W436(A)

UNIVERSITY OF SHEFFIELD

ELECTRON MICROSCOPY TECHNICIAN (Grade 5)

required for the

DEPARTMENT OF HUMAN BIOLOGY & ANATOMY

Applicants should have experience in the operation, and at least basic maintenance of transmission electron microscopes. Experience in biological specimen preparation, while valuable, is not essential. Salary on scale £3,700 to £4,320 p.a.

Please send details of qualifications, experience and the names of two referees to the Administrative Officer (Personnel), (Ref. S1362/N), or write informally to Professor R. Barer, Department of Human Biology and Anatomy, the University, Sheffield S10 2TN. 3401(A)

THE UNIVERSITY OF NEBRASKA-LINCOLN

BIOCHEMIST

Postdoctoral position available December 1 for research on the genetic control of cell differentiation. Our system utilizes 8 mutants affecting pigment production in cultured chick embryo pigment cells. Electrophoretic approaches will be used including PAGE, iso-electric focusing and 2-D separations. Previous experience in electrophoretic techniques required.

Appointment renewable annually with beginning salary $13.000. Send curriculum vitae and three letters of recommendation by November 1 to: Dr John Brumbaugh, School of Life Sciences, The University of Nebraska-Lincoln, Lincoln, Nebraska 68588.

Affirmative Action/Equal Opportunity Employer. W403(A)

Reactor Physics

We require a Research Officer whose main concern will be reactor safety studies with the CEGB Regional Scientific Services laboratories at Bristol.

Applicants should be in their mid-twenties with a good Honours degree in a science discipline. Experience in reactor physics is not essential, but candidates must have an appreciation of reactor plant construction and operation. Candidates will need to be able to run computer programs, compare and develop methods of analysis, interpret the results, report their findings lucidly, and stimulate whatever action is necessary in order to improve the performance of Magnox and AGR plants in the South Western Region. On the job training will be available where appropriate.

Salary will range from £5530, and may be on a scale rising to £8950 if you have sufficient relevant experience. Working conditions are excellent in newly built laboratories.

To apply, please write or phone for application form AF/1 from the Personnel Manager, Bedminster Down, Bridgwater Road, Bristol BS13 8AN. Telephone Bristol 648236 (during office hours). Forms should be completed, quoting reference P463/79/N, and returned by 5th November, 1979.

Central Electricity Generating Board
South Western Region

3479(A)

THE NATIONAL INSTITUTE FOR
HIGHER EDUCATION, LIMERICK

APPLIED MATHEMATICS LECTURER AS4 – 061

Applicants should have special competence in the area of numerical analysis and demonstrated expertise in the applications of mathematics. The person appointed will contribute to the development and operation of existing and new courses to degree and post-graduate levels.

A post-graduate qualification in a relevant area is desirable.

Salary Scale: £8,119 to £10,366 per annum.

Application forms, available from the Personnel Office, The National Institute for Higher Education, Limerick, Ireland, should be completed and returned by *2nd November, 1979.* 3485(A)

THE UNIVERSITY OF NEW ENGLAND

Armidale, New South Wales

DEPARTMENT OF AGRONOMY & SOIL SCIENCE

LECTURESHIP IN SOIL SCIENCE

The Division of Soil Science within the Department of Agronomy and Soil Science is responsible for undergraduate teaching of Soil Science in the Faculties of Rural Science, Resource Management, Arts and Economic Studies. Research and postgraduate training within the Division is concerned with various aspects of soil chemistry, biology and physics in relation to soil genesis, fertility and management.

The appointee will lecture and organise practical classes in introductory Soil Science courses. Supervision of advanced student projects will also be required. An appointee is sought in the areas of Soil Biology and/or Soil Management (soil conservation, pollution, reclamation, etc.) and preference will be given to persons in either of these areas having training in Soil Science and experience in teaching and research. The appointee is expected to take up duties in February 1980.

Salary: $A16,291 to $21,402 p.a. Assistance will be given with travel and removals.

Applicants in the UK, Europe and America should forward their applications, and ask their referees to forward their reports, without delay to the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, from whom conditions of appointment can be obtained. An additional copy of application only should be sent to the Staff Officer, University of New England, Armidale, NSW 2351, Australia, quoting Position No. 424.

Applications close 31 October 1979. 3496(A)

AGRICULTURAL RESEARCH COUNCIL

FOOD RESEARCH INSTITUTE

Scientific Services & Development Division

CHEMIST/PHYSICIST

A Scientific Officer is required to join a team of three in the Mass Spectrometry Group to use different types of MS and GC/MS for the analysis of samples sent to the Group in its role as the central ARS Mass Spectrometry Service.

A pass degree or HNC in chemistry or physics is required together with knowledge and practical experience of mass spectrometry and high resolution gas chromatography.

Salary:
From 1st August 1979: on a scale £3,289 to £5,085.
From 1st January 1980: on a scale £3,591 to £5,486.

Five-day working week and flexible working hours scheme operated. Transport facility available mornings and evenings.

Further particulars and application form from the Secretary, Food Research Institute, Colney Lane, Norwich NR4 7UA quoting reference 79/13. Closing date: 1st November 1979. 3512(A)

LEICESTER POLYTECHNIC

Faculty of Science

SCHOOL OF PHARMACY

LII/SL IN PHARMACEUTICAL CHEMISTRY

(Post No 564)

Applicants should be honours graduates in Pharmacy or a related discipline, preferably with a higher degree. Teaching responsibilities principally in the area of medicinal chemistry. Good research facilities available. Salary: £4,470 to £7,701 (bar) — £8,253 + £72 supplement p.a.

Further details and application forms available from Staffing Officer, Leicester Polytechnic, PO Box 143, Leicester, LE1 9BH. Tel (0533) 551551 Ext 2301/3.

3500(A)

BLOOD GROUP REFERENCE LABORATORY

SCIENTIFIC OFFICER

This laboratory is a Central Referenc
Laboratory for the National Bloo
Transfusion Service. A graduate is sough
for research and development o
techniques for the detection of antibodie
to neutrophil leucocytes. Experience i
immunology or immunohaematolog
desirable.

Application in writing with details c
experience and the names of two referee
to: Miss K. Lomas, Blood Grou
Reference Laboratory, Gatliff Road, o
Ebury Bridge Road, London SW1W 8QJ

3439(A)

UNIVERSITY OF SIERRA LEONE

Njala University College

Applications are invited for the post of

PROFESSOR/ASSOCIATE PROFESSOR IN CROP PROTECTION

in the Department of Agronom
Candidates should have a PhD preferab
in the area of Agricultural Entomolog
Experience in research and teaching i
tropical Institutions of Higher Learnir
would be an advantage.

Salary Scales: Professor Le8008 -
Le9,240 p.a. Associate Professor Le7,8
p.a. (£ sterling = Le2.25). The Britis
Government may supplement salary i
range £10,416 to £11,574 p.a. (sterling) f
married appointee or £7,758 to £8,910 p.
(sterling) for single appointee (reviewe
annually and usually free of all tax) an
provide children's education allowanc
and holiday visit passages. FSSU. Vario
allowances; family passages; bienni
overseal leave; part furnishe
accommodation at reasonable rental.

Detailed applications (2 copies) wit
curriculum vitae and naming thre
referees, to the Secretary, University
Sierra Leone, Freetown, Sierra Leone by
November 1979.

Applicants resident in the UK shoul
also send one copy to Inter-Universit
Council, 90/91 Tottenham Court Roa
London W1P 0DT. Further particula
may be obtained from either address.

3537(A)

THE MIDDLESEX HOSPITAL MEDICAL SCHOOL

Courtauld Institute of Biochemistry

Applications are invited for a

POSTDOCTORAL RESEARCH ASSISTANT

to work on the effect of pineal glan
methoxyindoles on other endocrine gland
Experience in working with glands an
isolated cells, HPLC, radioistopes woul
be an advantage. The post is for 3 yea
(commencing as soon as possible). Startin
salary according to age and experience u
to £5,650 per annum inclusive of £740 p
annum London Allowance.

Applicants should send curriculum vita
with names of 2 referees to Dr Ivor Smit
Courtauld Institute of Biochemistry, Th
Middlesex Hospital Medical Schoo
London W1P. 3514(A)

CELL BIOLOGIST/ BIOCHEMIST

Active research group using cell cultu
model systems to study mechanisms
hydrocarbon carcinogenesis and the effec
of tumor promoters on cell differentiatio
has two openings for Post-doctoral Fello
and Associate Scientists. Strong researc
background with experience in cell biolog
biochemistry and/or chemical carcin
genesis *in vitro* is required.

Please send curriculum vitae with nam
of three referees to Dr Leila Diamond or
Giovanni Rovera, Wistar Institute
Anatomy and Biology, 36th Street
Spruce, Philadelphia, PA 19104.

W446(A)

NMR POSTDOCTORAL POSITION

Applications are invited for postdoctoral work on the NMR of systems of chemical and biological interest, under the direction of Professor Eric Oldfield. Fourier transform spectrometers equipped with widebore superconducting magnets, operating at 360, 220 and 150 MHz, are available for use on the following projects: 1) Deuterium NMR of specifically ^{2}H-labelled membranes. 2) Phosphorus-31 NMR studies of protein-lipid interaction in model and biological membrane systems. 3) High-resolution ^{13}C and ^{2}H NMR spectra of proteins in solution and in the crystalline solid state. 4) NMR studies of metal ion nuclei in solution and in the solid-state, with special emphasis on systems of biological importance (such as ^{57}Fe, ^{67}Zn).

Applications consisting of a curriculum vitae, list of publications and letters from three references should be sent to Professor Eric Oldfield, School of Chemical Sciences, University of Illinois at Urbana, Urbana, Illinois 61801, USA. The University of Illinois is an Equal Opportunity/ Affirmative Action Employer. W449(A)

OCEANOGRAPHY CHAIRMAN

Applications are invited from persons with an earned doctorate, teaching, and research experience. Administrative experience is desirable, but not mandatory. Starting date January 1, 1980 or August 1, 1980.

Old Dominion is a state university. The Oceanography Department offers programs leading to the MS and PhD degrees with concentrations in biological, chemical, geological, and physical oceanography, as well as some undergraduate service courses. The departmental emphasis is on research and graduate teaching. The department has at present 10 faculty and 110 students. From 2 to 4 additional faculty may be added in the next three years.

Applicants should submit a curriculum vitae and a list of three references before November 15, 1979, to Professor Chester E. Grosch, Department of Oceanography, Old Dominion University, Norfolk, VA 23508. An Affirmative Action/Equal Opportunity Employer. W445(A)

STUDENT/JUNIOR TECHNICIAN

equired in busy Clinical Pharmacology Department, to assist in the general unning of the laboratories. Duties will nclude the care of instruments and quipment, ordering and checking of upplies and participation in the research ctivities of the department.

Knowledge and experience of general aboratory equipment essential. Day elease granted for relevant studies. Salary 2,235 to £3,501 plus £352 LW according to ge and qualifications.

Apply to the Secretary, Cardiothoracic nstitute, Fulham Road, London, SW3 HP, stating age, qualifications, xperience and the names of two referees. lease quote ref CP1/79. 3508(A)

POST DOCTORAL SCIENTIST is equired to fill a vacancy in the Radio-herapy Research Department of the nstitute of Cancer Research. The work of he Department involves the study of the iological effects of radiation and ytotoxic drugs on mouse tumours and on uman tumour xenografts, making use of issue culture techniques to assess *in vivo* esponses. The specific project is the levelopment of *in vitro* cell cloning echniques for the direct testing of chemo-ensitivity in human tumours and familiarity vith tissue culture and pharmacology vould be an advantage. The appointment vill be for an initial period of 3 years, with a ossibility of extension. Starting salary in ange £4,261 to £5,332 p.a. plus London llowance of £502 p.a. Applications in luplicate with the names of two referees to he Secretary, Institute of Cancer esearch, 34 Sumner Place, London SW7 NU, quoting ref 300/G/18. 3526(A)

UNITED KINGDOM ATOMIC ENERGY AUTHORITY

PHYSICISTS AND MATHEMATICIANS

are needed by the Safety and Reliability Directorate at Culcheth, Warrington to work in multi-disciplinary teams on a variety of technical studies and research on the safety of all types of reactor systems, but with special emphasis on fast reactor problems. Theoretical studies of reactor behaviour under fault conditions are carried out in association with relevant experimental studies undertaken at other establishments. The work involves Hydrodynamics, heat transfer, reactor physics, computing and many branches of classical physics, and the overall aim is to establish safety philosophy through identification of important problems, followed by their resolution in ways that can be applied to reactor design and environmental planning. In following this aim there is an extensive programme of collaboration with many overseas countries.

Applicants of either sex, should have a clear interest in reactor safety technology, or in the related field of reliability technology. The demands of the work are appropriate to people with academic abilities appropriate to good honours degree standard. A short introductory period will be allowed, but candidates will be expected to demonstrate at an early date their abilities for tackling the problems involved.

Appointments will be in the Science Group, Higher Scientific or Senior Scientific Officer levels. Applicants should normally have one of the following qualifications:

(a) a degree in a Scientific, Engineering or Mathematical subject
(b) degree standard membership of a professional institution
(c) HNC or HND in a Scientific, Engineering or Mathematical subject
(d) alternative qualification equivalent to at least (c) above.

For Higher Scientific Officer appointments, applicants with a 1st or 2nd class honours degree and a minimum of 2 years post graduate experience may be considered. Other applicants with alternative qualifications and at least 5 years appropriate post qualification experience, are not necessarily excluded from consideration.

For Senior Scientific Officer appointments applicants should normally have a 1st or 2nd Class honours degree, with a minimum of 4 years approved post graduate experience.

Rates of Pay as from 1 January 1980

Higher Scientific Officer	£5,455 — £7,210 p.a.
Senior Scientific Officer	£6,780 — £9,315 p.a.

Starting salaries within the scales may be enhanced to allow for good honours degree and/or appropriate postgraduate experience.

At least four weeks annual holiday, plus public holidays: Contributory Superannuation Scheme: five day week.

Write for application form to:

Personnel Officer
United Kingdom Atomic Energy Authority
Safety and Reliability Directorate
Wigshaw Lane
Cuclheth, Warrington, WA3 4NE

Quoting reference B44/J14

Closing date for the return of application forms: 7 November 1979.

3482(A)

CONTINENTAL PHARMA

DRUG METABOLISM AND PHARMACOKINETICS

A vacancy has recently arisen for a

GRADUATE SCIENTIST

to join the Drug Metabolism Section of
CONTINENTAL PHARMA'S RESEARCH CENTRE.

The work involves the study of absorption, distribution, metabolism and excretion of potential drugs in animals and man. Experience in experimental design, development of new bioanalytical methods and computer handling of the data is emphasized. The section is well equipped for liquid scintillation counting, gas-liquid and high pressure chromatography and mass-spectrometry.

The *ideal candidate* will be a senior graduate scientist with experience in the relevant field in industry or university. Such a candidate with adequate knowledge and qualifications might be asked to take charge of the expanding unit of Drug Metabolism and Pharmacokinetics.

A working knowledge of French in an asset.

The Research Centre is situated within easy reach of Brussels but will soon be moved to pleasant surroundings in the country. 20 miles from Brussels.

Written applications, giving details of qualifications and experience, should be addressed to:

DIRECTOR BIOLOGICAL RESEARCH
CONTINENTAL PHARMA
RESEARCH LABORATORIES
B-1830 MACHELEN (Bt), BELGIUM

W451(A)

KATHOLIEKE UNIVERSITEIT NIJMEGEN

postdoctoral fellow

for participation in studies on Drosophila spermiogenesis required. The applicant should be experienced in Drosophila genetics. He will be engaged in genetic studies dealing with Y chromosome function in D. hydei. Some participation in teaching is desired.

Salary according to qualifications between Dfl. 2.967,- and Dfl. 4.441,- per month.

Applications should be sent to Personnell Department, Faculty of Science, University of Nijmegen, Toernooiveld, 6525 ED Nijmegen.

Those who want further information before officially applying should write to the same address.

faculty of science
department of genetics
university of nijmegen
the netherlands

W438(A)

Queen Charlotte's Maternity Hospital

Goldhawk Road, London W.6.

This world famous Post Graduate Teaching Hospital has 161 beds and a high standard of patient care. It is situated within easy reach of shopping facilities at Hammersmith and Chiswick and is close to Stamford Brook tube station. A Day Nursery is available to employees children aged 2-5 years.

BASIC GRADE SCIENTIFIC OFFICER

Department of Microbiology

Applications are inivited from graduates in Nursing or Biological Sciences for the above post which is a Postgraduate Studentship. Previous experience is not essential. the successful candidate will be expected to supervise, develop and work in the Human Milk Bureau maintaining the Bank and to pursue a course of study leading to the research degree of Ph.D. in the University of London. This special Postgraduate Studentship is offered for 4 years.

Applications with names of two referees to Professor R Harley at the above address. 3518(A)

Scientist Engineer

New research department involved in the industrial applications of genetic engineering requires a Post Doctoral level scientist, experienced in Cloning Technology, DNA Biochemistry (including sequencing) Molecular Biology and Microbiology.

This permanent job includes important responsibilities.

Applications with curriculum vitae and requested salary should be sent to: Monsieur le Chef du Department du Personnel, Institut Pasteur Paris, 28 Rue du Docteur Roux, 75724, Paris, Cedex 15, France. **3498(A)**

FRITZ-HABER-INSTITUT DER MAX-PLANCK-GESELLSCHAFT

POSTDOCTORAL RESEARCH ASSOCIATE

(approx. DM 52.000, -- per annum). Two-year appointment, extendable, for biophysicist with interest in 3-D reconstruction of biological structures to collaborate in research project "Structure Function and Biosynthesis of Peptides and Proteins".

Experience desirable but not conditional. Write to:

Fritz-Haber-Institut der Max-Planck-Gesellschaft, Attn: Mrs. L. Lambert, Faradayweg 4-6, D-1000 Berlin 33 (West). W450(A)

UNIVERSITY OF LONDON

THE SOPHIA JEX-BLAKE CHAIR OF PHYSIOLOGY

TENABLE AT THE
ROYAL FREE HOSPITAL
SCHOOL OF MEDICINE

The Senate invites applications for the above Chair. Applications (10 copies) should be received by the Academic Registrar (214920N), University of London, Malet Street, London WC1E 7HU, from whom further particulars should first be obtained.

Closing date Tuesday 20 November, 1979. 3523(A)

GENETICIST: To conduct research in molecular genetics of human or other vertebrates and to teach molecular and human genetics. The level of appointment is at the Assistant or Associate Professorship and will depend on qualifications and experience. Interested persons should apply with complete curriculum vitae, including a statement of research and teaching interests, plus the names and addresses of three references.

Applications will be accepted through December 31, 1979 and should be addressed to: Geneticist Search Committee, Department of Genetics, University of California, Davis, California 95616. The University of California is an equal opportunity and affirmative action employer and invites applications from all qualified individuals. W439(A)

UNIVERSITY OF LEICESTER

DEPARTMENT OF BOTANY

Secondary product production by plant tissue cultures

A vacancy exists for a

RESEARCH TECHNICIAN

post in association with the above project. The post will be a Grade 3 limited tenure (two years) position on the following salary scale: £3,122 to £3,553. Candidates with appropriate qualifications and, if possible, experience in plant cell or tissue culture should apply to Professor H. Smith, Botany Department, University of Leicester, Leicester LE1 7RH. 3433(A)

Toxicology and Laboratory Staff

We are committed to develop our already substantial efforts in Toxicology and Occupational Health. These are devoted to the protection of our customers, our employees and the environment.

To support the planned programme of work, we have a number of posts for Toxicologists, Assistant Toxicologists and Laboratory Technicians. These will be based in a new, purpose built, fully equipped building on the Western outskirts of London.

For the posts of **TOXICOLOGISTS**, applicants should have the necessary qualifications and experience to make an immediate contribution to the work of a specialist team, including the supervision and training of junior staff.

Applicants should have:
✻ a science degree with preferably a post graduate qualification.
✻ proven ability to give sound professional advice at senior level on a wide range of toxicological issues.
✻ a successful record of leading work in one or more areas of experimental toxicology.

Applicants should be familiar with:
✻ the critical assessment of scientific data.
✻ the identification and solution of applied biological research problems.
✻ the application of toxicological principles to the regulation and control of chemical exposures.

For the more junior posts of **ASSISTANT TOXICOLOGISTS**, applicants should have the necessary qualifications and experience in applied biology to enable them to:
✻ give immediate help to senior colleagues in the production of critical data reviews.
✻ design and perform experimental studies.
✻ advise on the safe manufacture and use of a wide variety of products.

Applicants should have:
✻ a science degree.
✻ a knowledge of bio-medical effects of chemical substances.
✻ an interest in a full career in toxicology, based on a firm foundation of in-service training.

For the TOXICOLOGIST posts, applicants should be aged about 30 to 40 years. For the ASSISTANT TOXICOLOGIST posts, applicants should be aged about 22 to 28 years.

For the posts of **LABORATORY TECHNICIANS**, applicants should be able to assume immediate technical responsibilities in a biological research laboratory.

They should have experience in:
histo-pathology haematology
bio-chemistry animal husbandry techniques

Applicants should have science 'A' levels, be qualified to HNC level in medical laboratory technology (or be studying for such a qualification). They should be interested in pursuing a career in experimental toxicology and have a flexible approach to in-service work and training.

BP

Starting salaries will depend on qualifications and experience. There are excellent benefits which include a non-contributory pension scheme, excellent staff restaurants, sports and social facilities.
Please write for an application form which, for the TOXICOLOGIST posts, can be supplemented with a curriculum vitae, to:
Sue Bartholomeou, Central Recruitment,
The British Petroleum Company Ltd.,
Britannic House, Moor Lane, London EC2Y 9BU.

3534(A)

STANFORD UNIVERSITY

GEOCHEMIST —PETROLOGIST

The Department of Geology in the School of Earth Sciences invites applications for a faculty position beginning September, 1980. Areas of interest include (but are not imited to) geochemical processes in the crust and mantle of the earth including sotope geochemistry, thermodynamics, kinetics, element distribution and phase equilibria studies. Teaching responsibilities might include graduate and undergraduate courses in geochemistry and igneous petrology. Level of appointment is open, but we are particularly interested in a creative young person, with a well-established record in research.

Applications should include (1) a resume of education, professional experience, and published research; (2) a brief statement of plans for future research, and (3) the names of 4 referees. Send to: Prof. J.G. Liou, Department of Geology, Stanford University, Stanford, CA 94305, by December 1st, 1979. Stanford University is n equal-opportunity/affirmative-action mployer. W329(A)

BLOOD GROUP REFERENCE LABORATORY

SENIOR SCIENTIFIC OFFICER

The Blood Group Reference Laboratory is a Central Reference Laboratory for the National Blood Transfusion Service. This laboratory is a substantial producer of blood grouping reagents of human and animal origin. In the near future significant changes in manufacturing procedures are foreseen with changes in processing, the use of hyperimmune donors and *in vitro* cultures of lymphocytes as new sources of material. It is anticipated that this laboratory will be increasingly involved in monitoring quality of grouping reagents from a variety of external sources.

A Senior Scientific Officer is sought to take charge of all aspects of production and quality control of blood grouping reagents and to be responsible for new developments and research in these areas. Salary: £8,182 to £10,097 (increase pending) plus £502 L.W. Further information may be obtained from Dr A.M. Holburn, 01 730 2152.

Application in writing with details of experience and the names of two referees to: Mis K Lomas, Blood Group Reference Laboratory, Gatliff Road, off Ebury Bridge Road, London SW1W 8QJ.

3440(A)

HEAD OF PHARMACOLOGY DEPARTMENT

A leading Spanish Pharmaceutical Company situated in the mediterranean city of Barcelona invites applications from pharmacologists for the above post in a Pharmacology Department. The man we appoint will have a good honours degree in an appropriate discipline, a PhD in pharmacology and several years post-doctoral experience preferably in the pharmaceutical industry.

The successful applicant will be responsible to the director of Biological Sciences and must be able to formulate and organise research and development projects and collaborate with the other departments in the Biological and Chemical science groups.

Knowledge of Spanish is not initially essential but the successful candidate must be prepared to acquire adequate fluency in this language within 2 years. The salary is of US$40,000 pa, tax free and special assistance will be given for removal expenses to Barcelona.

Applications in writing with full curriculum vitae should be addressed to:

Pharmacology Department
P.O.B. 1583
BARCELONA-24 (Spain)

W437(A)

CONTINENTAL PHARMA

CARDIOVASCULAR RESEARCH

A vacancy exists in the Biological Research Department for a

SENIOR CARDIOVASCULAR PHARMACOLOGIST/BIOCHEMIST

to be in charge of a group of two graduates and six technicians.

The work involves research and development of new cardiovascular drugs. Original research possibilities are offered in the field of platelet aggregation, thrombosis and hypertension.

The *ideal candidate* will be a Ph. D. in pharmacology/biochemistry with adequate experience in this particular field of research in industry or university.

A working knowledge of French is an asset.

The Research Centre is situated within easy reach of Brussels but will soon be moved to pleasant surroundings in the country, 20 miles from Brussels.

Written applications, giving details of qualifications and experience, should be addressed to

DIRECTOR BIOLOGICAL RESEARCH
CONTINENTAL PHARMA
RESEARCH LABORATORIES
B-1830 MACHELEN (Bt), BELGIUM.

W452(A)

Public Health Laboratory Service
Centre for Applied Microbiology & Research, Porton Down, Salisbury, Wilts

Chief Medical Laboratory Scientific Officer

The Vaccine Research & Production Laboratory has a vacancy for a *Chief Medical Laboratory Scientific Officer*. The successful candidate will participate in the development of a capability for smallpox vaccine production, quality control and storage. Research in viral vaccine development will be encouraged, especially in the area of sub-unit vaccines. Candidates must have experience in virology, biochemistry or immunology and will be Fellows of the IMLS. Candidates who have other suitable qualifications will be considered but will be appointed to a different grade with a similar salary scale. Salary £6,243 to £7,059 increasing to £6,729 to £7,809 on 1 January 1980. Whitley Council terms and conditions of service.

Further details of the post can be obtained from Dr J.B. Griffiths, Deputy Director, Vaccine Research & Production Laboratory. Tel 0980 610391.

Applications stating date of birth, qualifications, experience and publications and naming three referees should be sent to

Mrs M. Bushby, CAMR,
Porton Down,
Salisbury, Wilts SP4 0JG
by 23 November 1979.

PHLS

3487(A)

DIRECTOR
INSTITUTE OF MARINE RESOURCES
PROFESSOR
SCRIPPS INSTITUTION OF OCEANOGRAPHY

Senior scientist with experience in innovative investigations related to marine resources and management thereof, to lead research groups at Scripps and University of California, Davis; prepare budgets for University funds and assist in obtaining extramural funds administer statewide Sea Grant College Program; and teach graduate students.

Appointed by the President, University of California. Salary (state funded) to $50,000 for 11 months, depending on qualifications. Send vitae and names of references by 31 December 1979 to M.M. Mullin, A-008, IMR Search, University of California, San Diego, La Jolla, CA 92093. The University of California is an Equal Opportunity/Affirmative Action employer. W456(A)

UNIVERSITY OF EDINBURGH
DEPARTMENT OF ZOOLOGY
BIOPHYSICIST/PHYSICIST/ PHYSICAL CHEMIST, PhD

required now for elastic neutron scattering research on proteins, viruses, etc. in Edinburgh, Institut Laue-Langavin Grenoble and Harwell. Good practical/theoretical knowledge of x-ray/neutron scattering, on-line computer handling, etc. essential. The successful candidate will be paid in accordance with nationally agreed research staff scales.

Apply immediately with references and details of experience to Sir John Randall, FRS, Department of Zoology, University of Edinburgh, West Mains Road, Edinburgh EH9 3JT. Please quote Reference 5023. 3520(A)

UMIST

Applications are invited for two **Experimental Officers and One Senior Experimental Officer** posts in the Department of Chemical Engineering. The posts are tenable for two years and are associated with a research programme, funded by the European Economic Community, on the development of a liquid membrane separation process for the selective recovery of metals from ores and dilute solutions. Applicants should have a degree or equivalent in Chemical Engineering, Chemistry, Metallurgy or Physics. Research and/or industrial experience would be an advantage, particularly for the senior post.

Salaries will be in the range: Experimental Officer £3,775 to £7,521) Senior Experimental Officer £7,227 to £8,992) under review

Requests for application forms and further particulars quoting reference number CE/197/AI should be addressed to the Registrar, UMIST, P.O. Box 88, Manchester M60 1QD. 3533(A)

UNIVERSITY OF LONDON
INSTITUTE OF PSYCHIATRY
RESEARCH ASSISTANT

Applications are invited from recently qualified graduates in pharmacology, biochemistry or related disciplines to undertake research into the transmitter role of peptides in motor function.

The position is tenable for two years — Salary £3,775 plus £740 London weighting on Salary Scale Research 1B — £3,775 to £5,488. Application forms are available from The Secretary, Institute of Psychiatry, De Crespigny Park, London SE5 8AF quoting ref PGJ/N (Phone 01 703 5411 ext 228).

Interested persons may visit the Department by prior arrangement. Phone 01-703 5411 ext 245. 3524(A)

THE UNIVERSITY OF HULL

Applications are invited for the post of

TECHNICAL SUPERVISOR
(Grade 6)

for the new

WOLFSON INSTITUTE OF GERONTOLOGY.

The work will involve the general supervision and administration of the animal house and laboratory, under the Director, Professor J G Phillips. The post carries responsibility for providing a technical service, either by institute staff or by an agency agreement with the Zoology Department to which the Institute is closely linked. Qualifications required are HNC or equivalent and at least 10 years relevant experience.

Salary on the scale £4,243 to £5,068 per annum.

Applications, naming three referees, and giving full details of age, qualifications and experience should be sent to the Technical Staff Officer, University of Hull, HU6 7RX by 31 October. Further Particulars are available. 3517(A)

SEDIMENTOLOGIST/ STRATIGRAPHER
Assistant or Associate Professor of
GEOLOGY

tenure-leading, August, 1980. Requires PhD plus demonstrated research abilities. Salary minimum $15,000. Duties include teaching sedimentology, litho-statigraphy and introductory courses. Direct graduate research. Preference will be given to applicants in the field of terrigenous clastic sedimentology who will be able to interact with current faculty in clastics, paleontology and groundwater geology.

Apply by January 15 with resume, transcripts and names of three referees to: Chairman, Department of Geology, 433 Morrill Hall, The University of Nebraska-Lincoln, Lincoln, Nebraska 68588. May also secure additional information about position from this address or by telephoning (402) 472-2663. Affirmative Action/Equal Opportunity Employer. W442(A)

UNIVERSITY OF QUEENSLAND
Australia
LECTURER IN PHARMACY

Applicants must have pharmaceutical qualifications acceptable for registration within the State of Queensland and appropriate higher degrees and proven ability in Pharmaceutics. *19 November 1979.*

Salary: $A16,291 to $A21,401 p.a. Other Benefits: Superannuation, housing assistance, travelling and removal expenses and opportunities to undertake programmes of special studies.

Additional information and application forms are obtainable from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. 3515(A)

QUEEN MARY COLLEGE
(University of London)
DEPARTMENT OF CHEMISTRY
RESEARCH TECHNICIAN
Grade 6

required to take charge of organic technica services. Person with organising skill and broad practical knowledge of modern techniques and instrumentation required. HNC or equivalent sought. Salary scale £4,243 to £5,068 p.a. plus £780 p.a. London Weighting. 5 day week, 5 weeks annual leave, plus a week at Christmas and Easter including public holidays.

Letters only to Establishment Officer, C/T, Queen Mary College, Mile End Road, London E1 4NS, stating age, experience and qualifications. 3453(A)

NATIONAL COUNCIL FOR SCIENTIFIC RESEARCH

Applications are invited from suitably qualified persons for the following posts:-

1. Plant Genetist (Forest Trees)
2. Tree Physiologist
3. Natural Products Chemist
4. Nutrition Biochemist (Fermentation)
5. Environmental Chemist
6. Food Micro-Biologist (Fermentation)
7. Food Scientist/Technologist (Oils and Fats)
8. Material Scientist (Building Materials)
9. Pulp and paper Technologist — Research)
10. Food Engineer

QUALIFICATIONS: For posts from one to seven, PhD or equivalent with relevant research experience.

For posts from eight to ten, M.Sc or equivalent with relevant research experience or B.Sc with extensive research experience.

SALARY: Attractive salary is offered within the range of ZK5424 to ZK8232 per annum.

Applications giving names and addresses of at least three references should be addressed to

The Secretary General
National Council for Scientific Research
P O Box CH 158
Chelston
Lusaka
Zambia
C. Africa

W455(A)

DEPARTMENT OF CHEMISTRY

APPOINTMENT IN BIOCHEMISTRY

Applications are invited for a tenure track appointment at the assistant professor rank, preferably with specialization in the area of macro-molecular biochemistry, starting September 1, 1980. The appointment is subject to budgetary approval. The successful applicant will be required to teach graduate and undergraduate courses in biochemistry and to establish a vigorous research program.

The current base of the assistant professor rank is $19,720 per annum.

Simon Fraser University is situated in Greater Vancouver, British Columbia.

Applications, including curriculum vitae, a concise statement of proposed research and the names of three referees, should be sent by December 31, 1979 to: Dr C H W Jones, Chairman, Department of Chemistry, Simon Fraser University, Burnaby, B.C. Canada V5A 1S6.

W426(A)

THE INSTITUTE OF LARYNGOLOGY AND OTOLOGY

330/332 Gray's Inn Road
London WC1X 8EE

Applications are invited for the post of

GRADUATE RESEARCH ASSISTANT

DEPARTMENT OF AUDIOLOGY

The successful applicant will be engaged in the measurement of hearing and balance in controlled clinical trials on patients suffering from vertigo. These studies will be conducted in the associated Royal National Throat, Nose and Ear Hospital. He/she will also be expected to liaise with general practitioners who will be involved in the study.

Applications in writing, giving qualifications, experience, and the names of two referees, should be sent to Professor R Hinchcliffe at the Institute. 3525(A)

UNIVERSITY OF LIVERPOOL

ENVIRONMENTAL ADVISORY UNIT

Applications are invited for the post of

ADVISORY OFFICER

in the Environmental Advisory Unit, Department of Botany. The Unit provides an advisory service and applied research facilities for Local Authorities and Industry on a wide range of environmental/ecological problems. Major projects centre on the reclamation of derelict and contaminated land, air pollution and environmental impact studies.

Applicants should have a good degree in Biological Sciences and a minimum of three years post-graduate research experience related to solving environmental problems. Preference will be given to candidates holding a PhD. A clean driving licence is essential: a generous car user's allowance will be given.

The post is tenable initially for one year, renewable annually subject to satisfactory progress.

Salary range £3,775 to £5,488 per annum depending on age and experience.

Applications, together with the names of three referees, should be received not later than 9th November, 1979, by The Registrar, The University, P.O. Box 147, Liverpool L69 3BX, from whom further particulars may be obtained. Quote Ref: RV/816/N 3510(A)

RESEARCH TECHNICIAN for the Department of Physiology either at trainee level or qualified. The successful candidate will be involved in neurological work and will be encouraged to take an active interest in the research programme. Experience with histological or pharmacological techniques an advantage but not essential. Applicants at trainee level should have a minimum of four 'O' levels, including science subjects and those for a higher grade relevant qualification up to ONC in Medical Laboratory Sciences or equivalent. Salary scale £1,834 to £2,553 p.a. (trainee) £3,122 to £3,553 p.a. (qualified). Ref. 429/C/343. Application form from, Assistant Secretary, Personnel Office, University of Birmingham, PO Box 363, Birmingham B15 2TT. 3489(A)

INTERNATIONAL CROPS RESEARCH INSTITUTE FOR THE SEMI-ARID TROPICS (ICRISAT)

needs the following personnel, all to be based at headquarters near Hyderabad, India, unless otherwise indicated:

PROGRAM LEADER, FARMING SYSTEMS RESEARCH

The appointee will be required to provide administrative and scientific leadership to a multidisciplinary team of about 30 scientists and supporting staff in developing farming systems that will increase and stabilize agricultural production in the seasonally dry semi-arid tropics. Must be able to understand, conceptualize and articulate an holistic approach to agriculture and to research in farming systems. Qualifications: Doctorate with excellent academic record in agronomy, soil science, agricultural economics or agricultural engineering. At least 10 years experience in field research with some experience in developing countries. Good theoretical background, a demonstrated scientific ability through formal publications and significant administrative experience are desirable qualifications.

SOIL AND WATER SCIENTIST

The appointee will work with the Associate Director, Cooperative Programs in liaison with ICRISAT programs in Africa and elsewhere and assist in preparation and writing of research projects, project funding proposals and reports. The post will involve regular overseas travel and will include assessment of ongoing programs. Qualifications: Doctorate with an excellent academic record in soil and water engineering, soil science, or agricultural engineering, substantial formal training in hydrology; proven ability for team work with other scientists in interdisciplinary research programs. Experience in agricultural research pertaining to less developed countries of the semi-arid tropics, at least ten years post-doctoral research experience, and an ability to speak and write French would be advantageous.

SOIL PHYSICIST

Responsible within a multidisciplinary, farming systems research program for developing and conducting research to investigate major soil physical factors that limit crop production and resource use in the semi-arid tropics and develop methods suitable for their alleviation by farmers of limited means. Qualifications: Doctorate in soil physics or associated sciences or equivalent. Ten years experience in both theoretical and applied soil physical research. Ability to lead a team of scientists and to collaborate in interdisciplinary research. Experience in tropical or arid envrionments would be an advantage.

CROP PHYSIOLOGISTS

One each for groundnut and pulses (pigeonpea and chickpea), to plan and conduct physiological research in cooperation with a multi-disciplinary team of international scientists. Research will be problem-solving and field oriented, but requires knowledge of modern laboratory techniques. Qualifications: A doctorate with excellent academic record; demonstrated scientific ability through formal publications and relevant work experience after post-graduation.

PLANT VIROLOGIST

Responsible for planning and conducting research, in cooperation with an interdisciplinary team of scientists, on virus diseases of ICRISAT crops, (Sorghum, Pearl Millet, Chickpea, Pigeonpea, Groundnut), with special reference to groundnuts. Qualifications: Doctorate in Plant Pathology with ten years research experience in plant virology, preferably in legumes, as evidenced by publication/reports. Experience in electronmicroscopy and serological techniques would be a definite advantage.

PLANT BREEDER, GROUNDNUT

(To be based in Africa)

Responsible for planning and conducting research, in cooperation with a team of international scientists, to develop advance breeding materials for national and regional programs. Qualifications: Doctorate in Plant Breeding with five years research experience in plant breeding, preferably in groundnut, as evidenced by publications/reports. Experience in the semi-arid tropics and bilingual ability in English and French would be an advantage.

TRAINING OFFICER

Responsible for assisting in development of practically oriented training program and instruction related to SAT crop improvement and natural resource utilization, with emphasis on communication in both French and English. Qualifications: Doctorate or equivalent in genetics, plant breeding, soil science, or agronomy with experience in teaching; French as first language or demonstrated ability to speak and write fluently in French; proven ability for team work with other scientists in interdisciplinary research and training programs. Ten years' post-doctoral research experience, and experience in agricultural training and research pertaining to less developed countries of the SAT would be an advantage.

Salaries for all of above positions commensurate with qualifications and experience; excellent fringe benefits.

Send resume by December 15, 1979 to: Director, ICRISAT, ICRISAT Patancheru P.O., Andhra Pradesh, 502 324, India. W4545(A)

NATURAL ENVIRONMENT RESEARCH COUNCIL
INSTITUTE OF OCEANOGRAPHIC SCIENCES

Oceanographic Research in connection with the possible disposal of High Level Radioactive waste in the Deep Ocean

The Department of the Environment has commissioned NERC to carry out a substantial programme of research in connection with the Government's decision to explore oceanic disposal options for high level radioactive waste.

The work will be carried out by the Institute of Oceanographic Sciences and will be based at its Wormley, Surrey, Laboratory. It will consist of long term projects in many of the disciplines of oceanographic research. The projects will be supervised by the senior staff of the Institute and will be run in parallel with the present research programme.

Applications are invited for thirteen posts for scientists and specialists and specialist support staff. It is expected that most of them will be filled at the SO/HSO level by people who have recently achieved graduate or postgraduate qualifications, though there is some flexibility for appointment at a higher level for posts where substantial experience is particularly valuable.

The posts to be filled are: –

1. **GEOLOGIST/GEOPHYSICIST** – to work on the selection and survey of areas to be studied and on the evaluation of potential disposal sites.
2. **MARINE SEDIMENTOLOGIST/STRATIGRAPHER** – to work on the stability of sediments in the deep marine environment.
3. **GEOPHYSICIST/ENGINEER** – to measure the physical properties of deep-sea sediments both *in situ* and in the laboratory.
4. **GEOLOGIST** – to investigate the distribution of ice rafted rock debris in the North Atlantic over the past million years.
5. **GEOPHYSICIST** – to study the three-dimensional transport of heat within deep-sea sediments by conduction and convection of pore waters.
6. **GEOCHEMIST/GEOLOGIST** – to investigate the diagenesis and chemical and mineralogical composition of pelagic marine sediments from different oceanic areas.
7. **BIOLOGIST** – to join the team working on various aspects of deep sea benthic biology.
8. **ELECTRONIC/ACOUSTIC ENGINEER** – to support work on underwater acoustics, transponder navigation and float tracking, telemetry from and control of bottom instrumentation.
9. **DESIGN ENGINEER/HYDRO DYNAMICIST** – to assist senior designers in the Ocean Engineering Group on work arising from the Deep Sea Radioactive Waste Disposal Programme.
10. **GEOCHEMIST** – to undertake day to day responsibility for research into the composition of interstitial waters of pelagic marine sediments in order to elucidate the physico-chemical processes which determine this composition.
11. **PHYSICIST** – to take a leading part in an experimental study of the benthic boundary layer.
12. **COMPUTATIONAL PHYSICIST** – to develop numeric models of the water movements within the benthic boundary layer.
13. **A SCIENTIST** – to assist with scientific administration in IOS in connection with commissioned research.

Salary scales are as follows:

	Present Rates	*From 1 January 1980*
Scientific Officer	£3289 – £5085 p.a.	£3591 – £5486 p.a.
Higher Scientific Officer	£4727 – £6211 p.a.	£5097 – £6737 p.a.
Senior Scientific Officer	£5876 – £7864 p.a.	£6332 – £8705 p.a.

Starting salary may be above the minimum, according to qualifications and experience.

The Natural Environment Research Council is not a government department, but conditions of service are similar to those of the Civil Service and there is a non-contributory pension scheme.

Application forms are available from The Secretary, Institute of Oceanographic Sciences, Wormley, Godalming, Surrey GU8 5UB, tel. Wormley (042 879) 4141 extn 272. Please quote the numbers of the post(s) for which you would like to apply.

Closing date: 2 November 1979. 3480(A)

THE POLYTECHNIC OF CENTRAL LONDON

SCIENCE DIVISION

TECHNICIAN GRADE 4 (Two Posts)

Salary:- £3,747 to £4,233 inclusive of London Allowance

1. Animal House

An experienced person required to see to the daily requirements of an animal house dealing with small mammals, duties include cleaning and sterilising of equipment and rooms, feeding and care of animals, arranging breeding programmes and ensuring home office regulations are implemented.

2. Chemistry Section

To join a team of Technicians working in teaching and research laboratories, knowledge of chemical and biochemical techniques would be an advantage. Person appointed would be expected to prepare materials for practicals including preparing solutions and providing assistance to staff and students.

Qualifications and experience for both posts C&G Part I, ONC, OND or 2 'A' Levels with 7-9 years experience.

Application form and job description from the Establishment Officer, PCL, 309 Regent Street, London W1R 8AL (Tel. 01-580-2020 ext.212). 3502(A)

SHEFFIELD AREA HEALTH AUTHORITY (TEACHING)

CENTRE FOR HUMAN GENETICS

CYTOGENETICIST

There is an immediate vacancy for a Cytogeneticist (Basic Grade) at the above unit. The work is part of a Regional diagnostic service and will include tissue culture and the identification of chromosome abnormalities. Applicants should have a relevant Science degree.

Salary scale £3,486 rising to a maximum of £5,592. For further information telephone Sheffield (0742) 667333.

Applications giving details of age, qualifications and experience, with the names and addresses of two referees to: The District Personnel Officer, Floor B, Hallamshire Hospital, Glossop Road, Sheffield S10 2JF. 3507(A)

UNIVERSITY OF OXFORD

RESEARCH OFFICERSHIPS IN NUCLEAR PHYSICS

The University proposes to appoint two Research Officers in the Department of Nuclear Physics, in the field of experimental nuclear structure physics, for a period of three years, from 1 April 1980 or earlier if possible. Salary according to age on the University Lecturer scale (under review) £4,333 to £9,581. Further particulars may be obtained from the department.

Applications (one typed copy) including a statement of qualifications, publications and the names of two referees, should reach Professor K.W. Allen, Department of Nuclear Physics, Keble Road, Oxford OX1 3RH by 30 November 1979. 3488(A)

THE UNIVERSITY OF HULL

DEPARTMENT OF BIOCHEMISTRY

A vacancy exists on the established staff for

A SENIOR RESEARCH TECHNICIAN

Grade 5

The post is connected with the Departmental Chemostat and Fermenter Suite and the duties will be mainly concerned with research into the synthesis of lipid substances in Microorganisms. Minimum qualifications: HNC or equivalent, plus 8-9 years experience in Biochemistry. Additional experience in Microbiology, particularly in fermentation techniques, would be advantageous. Salary on the scale £3,700 to £4,320 per annum. Applicants with less experience will be considered, to commence initially on a lower grade. Ref. No. TBCH/5.

Applications giving details of age, qualifications and experience should reach the Technical Staff Officer, University of Hull, HU6 7RX by 31 October quoting the names of two referees. 3483(A)

CITY OF LONDON POLYTECHNIC

DEPARTMENT OF BIOLOGICAL SCIENCES

GRADUATE RESEARCH ASSISTANT

A graduate research assistant is required immediately to work on an Agricultural Research Council sponsored study of plant substances affecting calcium metabolism. The project will be of interest to biochemists/physiologists/pharmacologists. The successful applicant will be encouraged to register for a higher degree.

Salary: £3,321 to £3,417 to £3,513.

A full curriculum vitae giving the names and addresses of two referees should be sent to Dr. K.M.L. Morris, Department of Biological Sciences, Old Castle Street, London E1 7NT, within two weeks of the appearance of this advertisement. 3484(A)

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF SURGERY

Applications are invited for the post of post-doctoral University Research Assistant/Senior Research Assistant in the Department of Surgery, to become the scientific member of a clinical team investigating clinical, physiological and biochemical aspects of liver disease, particularly portal hypertension and oesophageal varices.

The appointment will be tenable for one year in the first instance and may be renewed though not normally more than twice. The initial salary will be within the range £3,775 to £5,488 per annum according to qualifications and experience.

Arrangements to visit the Department may be made with Professor R. Shields and Mr I Taylor, telephone 051-709 0141 ext 2676 and 2677 or 051-708 0020.

Applications, together with the names of two referees, should be received not later than 12th November, 1979, by the Registrar, The University, PO Box 147, Liverpool L69 3BX, from whom further particulars may be obtained. Quote ref RV/806/N. 3494(A)

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF ORTHOPAEDIC AND ACCIDENT SURGERY

TECHNICIAN (Grade 5)

Duties include responsibility for histological preparations and autoradiographs of bone, cartilage and connective tissues for the research and teaching programme into arthritis, fracture repair and bone cancer. Previous experience of photographic techniques an advantage.

Salary in a range £3,700 to £4,320 p.a. (under review) according to qualifications and experience.

Application forms may be obtained from the Registrar, The University P.O. Box 147, Liverpool L69 3BX. Quote Ref: RV/815/N 3509(A)

SEDIMENTOLOGIST/Stratigrapher, Assistant or Associate Professor of Geology, tenure-leading, August, 1980. Requires PhD plus demonstrated research abilities. Salary minimum $15,000. Duties include teaching sedimentology, lithostatigraphy and introductory courses. Direct graduate research. Preference will be given to applicants in the field of terrigenous clastic sedimentology who will be able to interact with current faculty in clastics, paleontology and groundwater geology. Apply by January 15 with resume, transcripts and names of three references to: Chairman, Department of Geology, 433 Morrill Hall, The University of Nebraska-Lincoln, Lincoln, Nebraska 68588. May also secure additional information about position from this address or by telephoning (402) 472-2663. Affirmative Action/Equal Opportunity Employer. W448(A)

THE UNIVERSITY OF MANCHESTER

DEPARTMENT OF ANATOMY

RESEARCH TECHNICIAN (Grade 5)

Applications are invited for the post of research technician to work in the area of experimental neurology. Applicants must hold HNC or equivalent and have at least 8 years' laboratory experience. Competence in a wide range of modern techniques in histology and microscopy including autoradiography is essential. Previous experience in research work is required and a working knowledge of the use of neuroanatomical, neurophysiological or neuropharmacological research techniques would be a considerable advantage.

Salary scale £3,700 to £4,320 pa together with further comparability payments and subject to overall review as from 1st October 1979.

Applications giving details of age, qualifications, experience and the names of two referees should be sent to Professor P.F. Harris, Department of Anatomy, Medical School, University of Manchester, Manchester M13 9PT. 3506(A)

ASSISTANTSHIPS

UNIVERSITY OF LEICESTER

DEPARTMENT OF BOTANY

Postdoctoral/postgraduate research assistantship in secondary product production by plant tissue cultures

Two posts are available for research on the above project to be held either at postdoctoral or postgraduate research assistant level. In the case of a successful applicant's being appointed at the post-doctoral level the appointment will be for two years only. In the case of a candidate's being appointed at postgraduate level funds will be available for three years and registration for higher degrees will be possible.

Further information on the project and related conditions may be obtained from Professor H. Smith, Botany department, University of Leicester, Leicester LE1 7RH. 3432(P)

UNIVERSITY OF DURHAM

GLYCOPROTEIN RESEARCH UNIT

Applications are invited for a

SENIOR RESEARCH ASSISTANTSHIP

tenable for one to two years, and for a

RESEARCH ASSISTANTSHIP

tenable for one year, in the Glycoprotein Research Unit. The work involved will be studies on the control mechanisms of biosynthesis and secretion of glycoproteins by human fibroblasts *in culture* and by chicken trachea *in vitro* with some emphasis on the role of calcium-binding proteins. It is expected that the candidate for the senior post should have experience at doctoral level in cell-culture and membrane fractionation techniques. Knowledge of isotopic-labelling and chromatographic separation methods will be essential for both posts. Initial salary for the Senior Research Assistant will be in the range of £4,333 to £5,199 p.a. and for the Research Assistant £3,775 to £4,333 p.a. plus superannuation.

Applications, including curriculum vitae and names of three referees should be sent by 31st October 1979 to the Registrar, Science Laboratories, South Road, Durham, DH1 3LE. For further information contact Dr P W Kent or Dr N Mian at Durham 64971, ext 239.

3527(P)

UNIVERSITY OF GLASGOW

DEPARTMENT OF CELL BIOLOGY

POST-DOCTORAL RESEARCH ASSISTANT

Applications are invited for a

RESEARCH ASSISTANTSHIP

funded by the M.R.C., to study the membrane properties of mononuclear phagocytes as related to their behaviour. The person appointed would work with a small group interested in the cellular aspects of inflammation; a background in cellular immonology or cell biology would be advantageous. The appointment is for a maximum of three years, starting as soon as possible, and would be on salary range 1A for Research and Analogous Staff (£4,333 to £5,777). Appropriate superannuation scheme will apply.

Applications, enclosing a curriculum vitae and the names of two referees, should be sent to Dr J.M. Lackie, Department of Cell Biology, University of Glasgow, Glasgow, G12 8QQ.

In reply please quote Ref. No. 4506/1M.

3511(P)

FELLOWSHIPS

UNIVERSITY COLLEGE DUBLIN

FACULTY OF AGRICULTURE

POST-DOCTORAL FELLOWSHIP

Applications are invited for a post-doctoral fellowship (sponsored by the National Board for Science and Technology) to study the micromorphology of structurally-damaged and unstable topsoils in Ireland.

Candidates must have considerable experience in soil micromorphology; competence in soil physical measurements is also desirable. The initial appointment is for one year with the probability of renewal for a further two years. The salary scale is approximately £4,200 to £4,800.

Applications, with details of qualifications and experience, to:

Dr J.F. Collins, Department of Agricultural Chemistry and Soil Science, Agriculture Building, Belfield, Dublin 4.

3516(E)

-Unique Opportunity-

STANFORD UNIVERSITY

NSF POSTDOCTORAL FELLOWSHIP in MOLECULAR BIOLOGY and COMPUTER SCIENCE

$14,000 per annum

Applicants should be familiar with the techniques of modern molecular biology and be interested in entering the field of computer science. Previous programming experience is useful but not essential. Responsibilities will include development and maintenance of a knowledge base of current laboratory techniques for use with an advanced model of automated experimental planning. One goal of this program is to train an individual for a career in computer science as applied to molecular biology and genetics. While the fellowship will be awarded through the Department of Computer Science, the fellow will interact regularly with research groups in the Departments of Biochemistry and Medicine.

Send resume and the names of three references to either:

Dr Douglas Brutlag, Department of Biochemistry or Dr L.H. Kedes, Department of Medicine, Stanford University, School of Medicine, Stanford, California 94305. Equal opportunity through affirmative action. W383(E)

POST-DOCTORAL FELLOW

Laboratory with active program in the study of hormone receptor/antibody interactions has an opening for an individual holding a PhD degree and with experience in hormone receptor separation and purification. Although funds for the position at the M.R.C. Canada Postdoctoral Fellowship rate are available, the successful candidate would be encouraged and assisted in applications for his/her own Fellowship support.

Applications should include curriculum vitae and previous research experience. The applicant should request three referees to send letters of support to: Dr. N.R. Farid, Faculty of Medicine, Health Sciences Centre, 300 Prince Phillip Drive, St John's, Newfoundland A1B 3V6. W441(E)

GIRTON COLLEGE CAMBRIDGE

Applications are invited for a scientific research fellowship open to graduates of any university and tenable for three years from 1st October 1980. The applications should be in the field of Mathematics, Natural Sciences, Geography and allied subjects.

The fellowship is to the value of £3,000 per annum to a candidate with a PhD and £2,750 per annum to a candidate who has not completed a PhD. The fellowship is pensionable under U.S.S.

Particulars are available from the Secretary to the Council, Girton College, Cambridge CB3 0JG to whom applications should be sent by 19th November, 1979.

3503(E)

NEWNHAM COLLEGE CAMBRIDGE

RESEARCH FELLOWSHIPS

The College, acting as part of a Group, invites applications from women for one or more Research Fellowships which will normally be tenable from 1st October 1980.

Further details, together with application forms (which must be completed and returned not later than 20th October 1979) may be obtained from the Secretary, C.I.A.O., Kellet Lodge, Tennis Court Road, Cambridge CB2 1QJ.

3528(E)

THE BRITISH COUNCIL

Academic visits to the Soviet Union, Czechoslovakia, Hungary, Romania, Bulgaria and German Democratic Republic

Applications are invited from British academic staff in all disciplines for short (two week) and medium term (1-6 months) visits, under agreed exchange programmes, to the above countries. Short visits normally involve a tour of a number of institutions appropriate to the visitor's subject area. Medium term visits are intended to facilitate research and are usually based at one establishment.

For details and application forms please write or telephone to: Higher Education and Science Department, The British Council, 10 Spring Gardens, London SW1A 2BN. Tel: 01-930 8466 ext: 2683.

Closing dates for completed applications is 10 November 1979.

Please quote 79/E2 when responding to this advertisement. 3531(E)

QUEEN MARY COLLEGE

University of London

PLANT BIOLOGY AND MICROBIOLOGY DEPARTMENT

Applications are invited for a Postdoctoral Research Fellowship sponsored by SRC Marine Technology Directorate for work on distribution and activity of Sulphate-Reducing Bacteria in North Sea sediments related to anoxic corrosion of mild steel. Experience of working with anaerobic bacteria an advantage. Appointment for 1 year in first instance, starting not later than January 1980.

Initial salary in range (under review) £5,073 to £5,650 p.a. (including London Allowance). Applications, including curriculum vitae and names of 2 referees, should be sent as soon as possible to The Secretary, Queen Mary College, Mile End Road, London E1 4NS. 3499(E)

STUDENTSHIPS

THE OPEN UNIVERSITY

SRC Case Studentship in Chemistry

Applications are invited for a Case Studentship, tenable from a mutually agreed date prior to 1st March 1980 and leading to the degree of PhD.

The project involves the development of synthetic routes to certain types of organic heterocylic compound that are of interest for their anti-inflammatory or anti-hypertensive activity. The syntheses are centred around a photochemical step, and there is scope for studying the mechanism of these photoreactions.

The supervisors are Dr John Coyle of the Open University and Dr Keith Godfrey of Reckitt and Colman (Pharmaceutical Division).

Candidates should possess, or expect to obtain, a good honours degree or its equivalent in either Chemistry or Combined Science with Chemistry as a major subject. Applications giving a full curriculum vitae and the names and addresses of two academic referees should be sent as soon as possible to *Dr J.D. Coyle, The Open University, Walton Hall, Milton Keynes, MK7 6AA* from whom further particulars can be obtained.

Please quote reference (CC/1). Initial enquiries may be made by telephone to Dr Coyle (Milton Keynes 653483). 3490(F)

UNIVERSITY OF OTAGO
Dunedin, New Zealand
NZERDC STUDENTSHIP

Applications are invited from persons with a good honours degree in Geology for work leading to a PhD on the tertiary sedimentary rocks of the Waiau Basin, a fault controlled basin at the southern end of the Alpine transform fault.

The project will involve preparation of a basin analysis with particular attention to hydrocarbon potential, and applicants should be able to perform field work in rugged terrain.

Further details and application forms may be obtained from the joint supervisor of the project Dr R M Carter or Mr R J Norris, University of Otago, Geology Department, PO Box 56, Dunedin, New Zealand. 3521(F)

UNIVERSITY COLLEGE OF SWANSEA

Case Research Studentship

Applications are invited from candidates with an interest in Organic Chemistry and with a good honours degree or equivalent for an S.R.C. **CASE Research Studentship** in the **Department of Chemistry.**

The award is for the study of the synthesis of novel macrocyclic lactones with potential central nervous system activity and will be in collaboration with Pfizer Ltd.

Letters of application, which should include the names and addresses of two referees, should be sent to Dr K Smith, Department of Chemistry, University College of Swansea, Singleton Park, Swansea, SA2 8PP, ***as soon as possible.*** 3535(F)

UNIVERSITY OF SOUTHAMPTON
RESEARCH IN CRYOGENICS
Superconducting AC Generators

Applications are invited for an
S.R.C. CASE Studentship

on "Refrigeration cycles for rotating helium at 3000 rpm". The successful candidate will be expected to register for a higher degree.

Applicants with a good degree in Science or Engineering should write to Dr R.G. Scurlock, Cryogenics Group, University of Southampton, S09 5NH. Please quote reference N. 3530(F)

THE OPEN UNIVERSITY

Research Studentship(s) in Biology/Brain Research

Studentships, leading to a PhD, available immediately to work with:

(1) Professor Steven Rose on environmental, nutritional and hormonal factors influencing developmental biochemistry of the brain.

(2) Dr Rob Random on developmental genetics of drosophila.

Candidates should preferably have 1st or 2(i) degrees in i) biochemistry or neurobiology (ii) genetics.

For further particulars, etc., telephone Professor Rose or Dr. Ransom on (0908) 653225 or (0908) 653464 as soon as possible. Please quote reference BR2. 3491(F)

UNIVERSITY OF SOUTHAMPTON
DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY

Applications are invited from holders of good honours degrees in Biochemistry, Pharmacology or Psysiology for a Duphar Research Studentship to study the mechanism of action of cerebral activating drugs. The terms and conditions of the award are equivalent to Research Council awards, and will include registration for a higher degree. The successful applicant will be required to start work as soon as possible.

Further details may be obtained from Dr N.M. Horn, Department of Physiology and Pharmacology, Medical and Biological Sciences Building, Bassett Crescent, East, Southampton, SO9 3TU, to whom applications including the names and addresses of two academic referees should be sent. Please quote reference N. 3493(F)

UNIVERSITY OF BIRMINGHAM
CASE Studentship
The Physical ageing of PVC and copolymers

Applications are now invited from suitably qualified Chemists, Physicists or Material Scientists for a three year SRC (CASE) Research Studentship in the Department of Chemistry. In collaboration with BP Chemicals Ltd, the programme will involve assessing the effect of physical ageing and morphology in the mechanical properties of PVC.

Applications, together with the names and addresses of two referees, should be sent to: Professor J.C. Tatlow, Department of Chemistry, University of Birmingham, PO Box 363, Birmingham, B15 2TT. 3486(F)

FOR SALE and WANTED

"FROM MASSLESS to Material Universe" by M.E. Winfield. Written in language understandable by scientists of all disciplines, the book presents a new outlook on the foundations of physics. It argues for the evolution of matter from particles simpler than any yet known, and offers suggestions as to the nature of the particles. The evolutionary hypothesis provides for the existence of fields more subtle than the electromagnetic and gravitational, and will therefore be of interest to scientists attempting to explain the Kirlian effect and related phenomena. Price $6.50 Australian. Available by mail order from Golden Point Press, Box 373, Lakes Entrance, Victoria 3909, Australia. W443(L)

CONFERENCES

NATO ADVANCED STUDY INSTITUTE
"ACTIVE DEFENCE MECHANISMS IN PLANTS"

This meeting will be held at Cape Sounion, south of Athens, Greece 21 April to 1 May 1980. It will be based on *c.* 15 lectures by well known authorities, papers and discussions on the active mechanisms by which higher plants prevent infection and colonization by microorganisms, viruses and related agents.

Attendance will be by invitation. Part of travel and hotel expenses may be met for participants from NATO countries who cannot be wholly supported from other sources. There will be a few places but no expenses for scientists from other than NATO countries.

Applications to attend should be sent as soon as possible to Professor R K S Wood, Botany Department, Imperial College, London SW7, UK, stating age, title of paper to be submitted, contribution to be made towards expenses of attendance. 3519(C)

Gordon Research Conferences

"Frontiers of Science"

1980 Winter Schedule, Miramar Hotel, Santa Barbara, California

January 7-11	Liquid Crystals	Sensory Transduction in Microorganisms
January 14-18	Polymers	Orientational Disorder in Crystals
January 21-25	Thin Organic Films and Solid Surfaces	Deformation Mechanisms in Polymers and Composites
January 28-February 1	Electrochemistry	Chemical Oceanography
February 4-8	Chemistry and Biology of Peptides	Physico-Chemical Aspects of Photosynthesis
February 11-15	Chemical Aspects of Plant-Herbivore Interaction	
February 18-22	Immunochemistry and Immunobiology	
February 25-29	Metals in Biology	Structural Macromolecules: Collagen

Conference Fees:

Conferee (registration, room, meals)	$215.00
Guest (room, meals)	$165.00

Program: The complete program will be published in the October 19, 1979 issue of *Science*.

Requests for applications or for additional information should be addressed to:

Dr Alexander M. Cruickshank, Director
Gordon Research Conferences
Pastore Chemical Laboratory
University of Rhode Island
Kingston, RI 02881, U.S.A.

W453(C)

An international conference on

Safety Evaluation of Nitrosatable Drugs and Chemicals

to be held at
The Royal College of Physicians, London, England
on
24th and 25th January 1980

To assess the extent of human risk from the nitrosation of drugs and chemicals in vivo. *To evaluate the methodology available for the safety evaluation of nitrosatable chemicals.*

Under the auspices of
The University of Surrey and Pharmaceutical Industry

Conference limited to 300 participants

For further particulars:
Dr. C. Ioannides,
Department of Biochemistry,
University of Surrey,
GUILDFORD,
Surrey, GU2 5XH, U.K.

3529(C)

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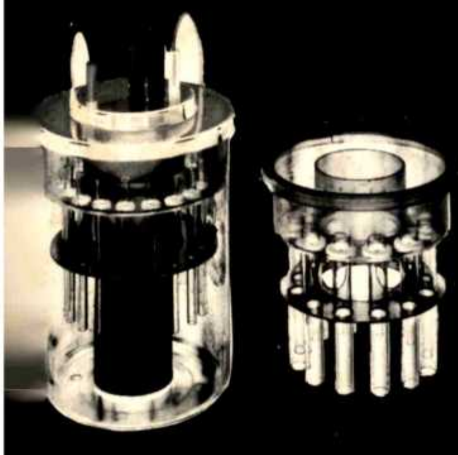
HO R
SCIENTIFIC
INSTRUMENTS

Specialists in
Gel Electrophoresis



New Tube Gel Unit.
Interchangeable upper buffer chambers serve 5 tube gel sizes. GT Series.

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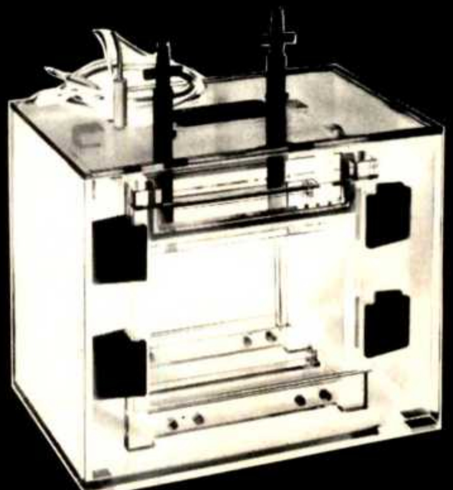
Slice up to 160 one mm segments in one pass—at room temperature. SL 280



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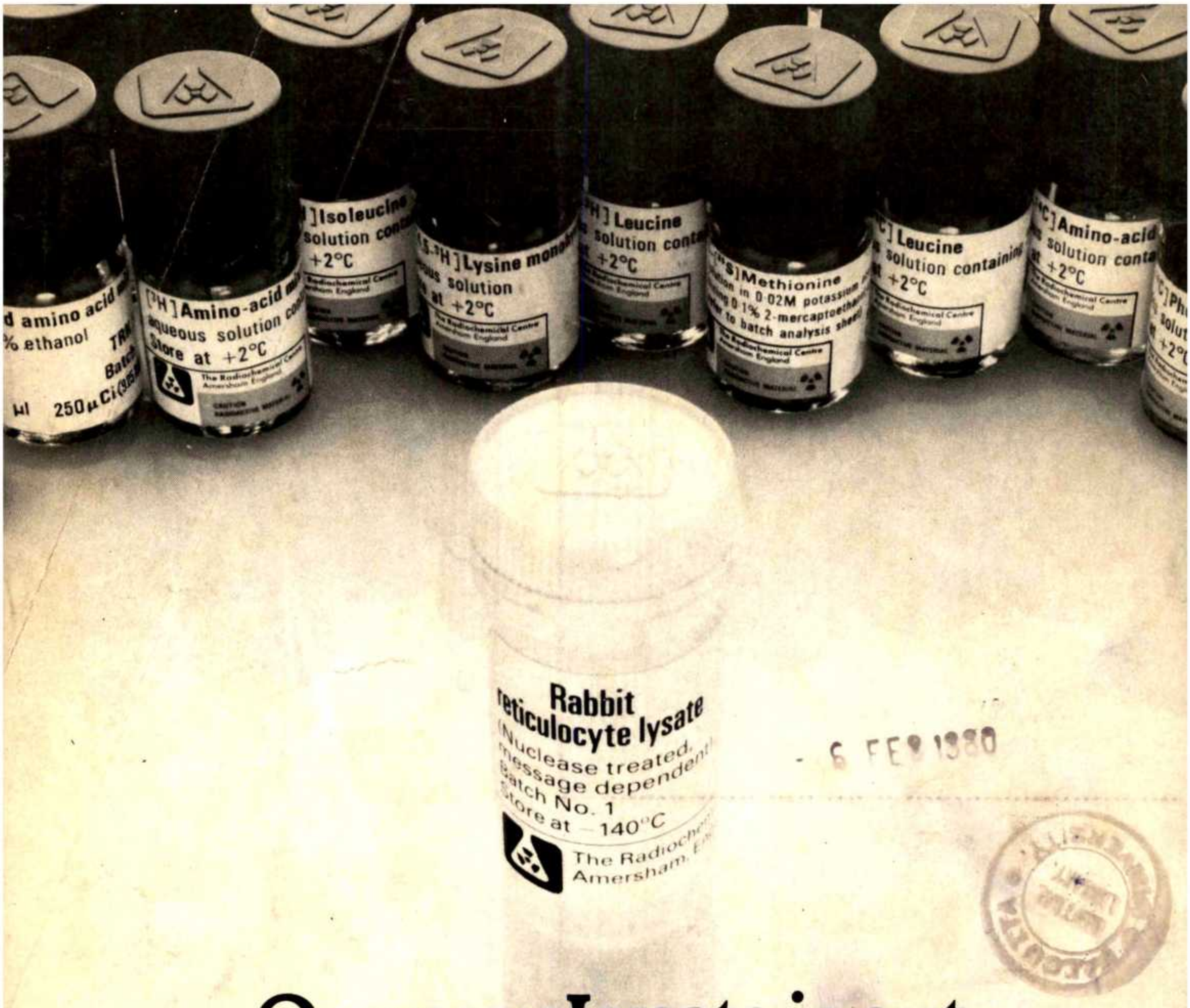

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* based on the method of Pelham, H.R.B. and Jackson, R.S. *Eur. J. Biochem.*, **67,** 247-257, 1976.

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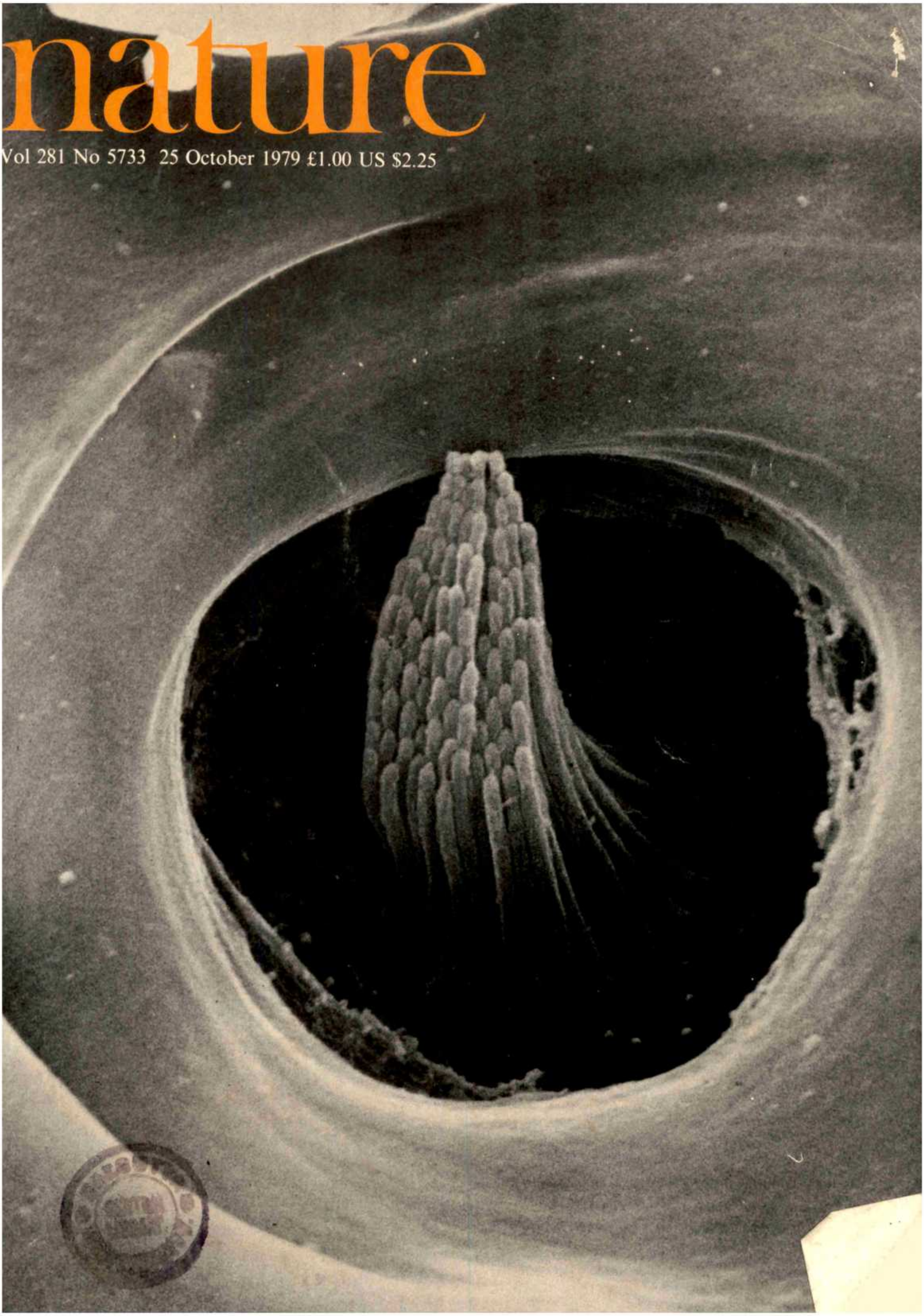

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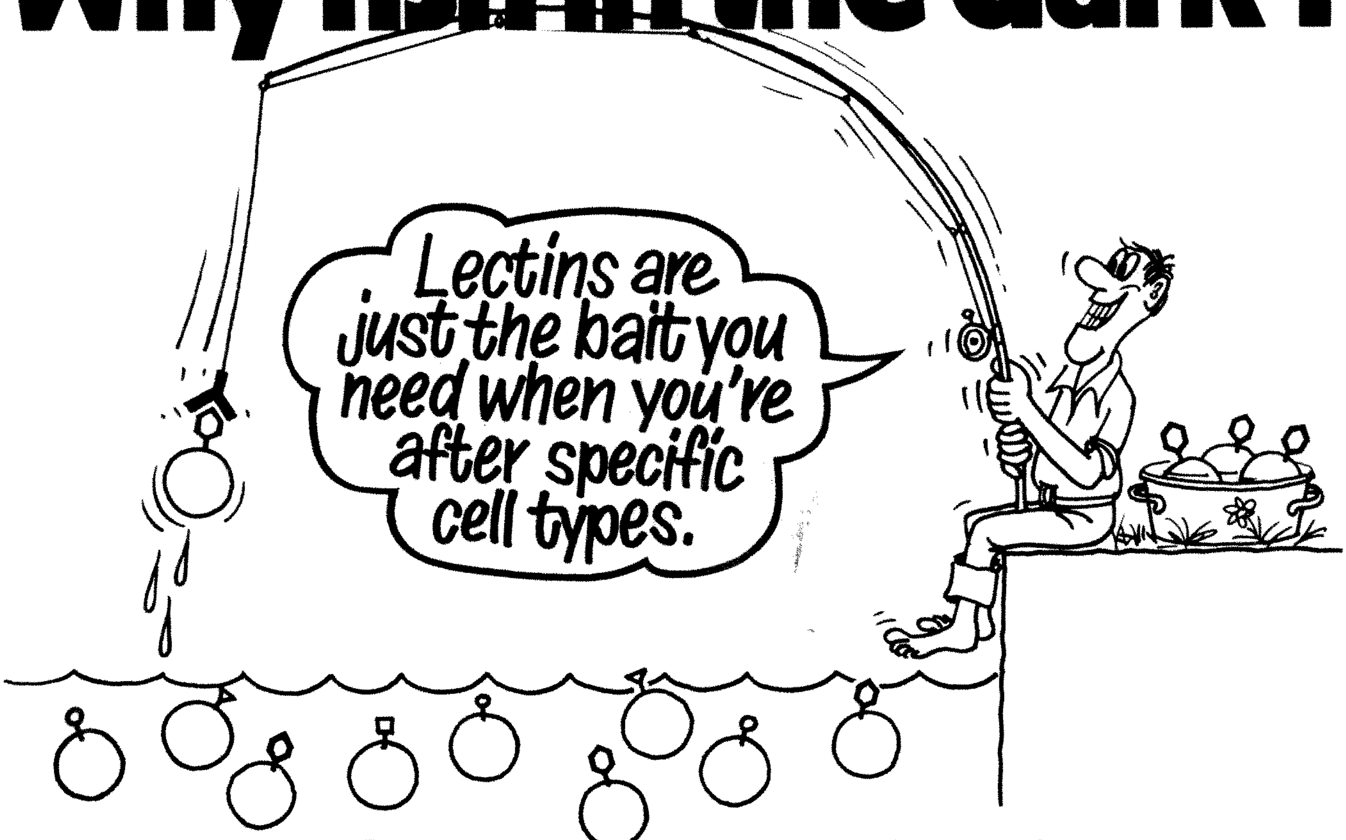
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Cover picture

Scanning electron micrograph of the apical surface of a hair cell from the bullfrog's inner ear, and of the otolithic membrane that surrounds it. The ionic currents responsible for transduction by such cells are described on page 675. (Micrograph courtesy of Mr R. Jacobs.)

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CUT YOUR AD BUDGET LATELY?

Honesty compels us to admit that you're not alone.

Whenever the cost/price squeeze gets really tough, it's a temptation to regard advertising as a cost... and to cut.

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In recent years, a significant change has taken place in the thinking of many management men about advertising budgets. No longer are appropriations cut automatically when the pressure is on.

Why?

For a number of reasons. Among them are:

1. *With the growth of the marketing concept,* advertising is no longer looked upon merely as an expense, but as an integral part of the company's marketing mix.

2. *Firms that maintain advertising during recession years do better in sales—and profits—in those and later years.* That was proved conclusively in studies of five separate recessions made by ABP and Meldrum and Fewsmith.

3. *The cost of a salesman's call today makes it imperative to make maximum use of advertising.* The average cost of an industrial sales call soared to a record $96.79 according to the latest report by McGraw-Hill's Research Laboratory of Advertising Performance. Yet studies show that a *completed* advertising sales call—that is, one ad read thoroughly by one buying influence—literally costs only pennies. Why deny yourself such efficiency?

4. *In some cases, there is no way to reach customers except by advertising.* The "Paper Mill Study" shows (1) the number of buying influences in the average plant is far greater than marketers are aware of, (2) the vast majority of these influences are unknown to salesmen, (3) no salesman has the time to contact all influences even if he knows them.

5. *Selling costs are lower in companies that assign advertising a larger role in marketing products.* So advertising is an investment in profit, just like a machine that cuts production costs.

6. *Memories are short.* There is an estimated 30% turnover every year among buyers. It isn't surprising, then, that lack of advertising contact can quickly result in loss of share of market.

7. *Most down periods turn out to be shorter than expected.* The history of every postwar recession is that it didn't last as long as predicted. Why gamble your market position for short-term gain?

8. *Consider lead time.* Very few products sold to business and industry are bought on impulse. The advertising you are doing—or missing—right now will have its effect years from now.

9. *Advertising works cumulatively.* It would be nice to think that every reader reads all of your ad. We know it doesn't work that way. To be most effective, advertising must have continuity.

10. *Did your competitor cancel his budget, too?* If not, you may be taking a big risk.

11. *Will you lose salesmen?* They know that their chance of getting an order is better if they are backed up by advertising. Can you be sure of keeping them when they learn that that support has gone?

12. *You know better.* Survey after survey of executives shows that they expect a *drop* in sales if advertising stops.

But there is need for efficiency...

whenever advertising budgets are being assembled—never more than in these inflationary times. Significantly, a recent survey shows that nearly 40% of the average budget for advertising to business and industry is invested in business publication space and preparation. That's *more than double* the next largest item.

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- Letters are brief reports of original research of unusual and wide interest, not in general longer than 1,000 words; they have at most three or four displayed items.
- 'Matters Arising' permits short discussion (up to 300 words) of papers that have recently appeared in *Nature*.

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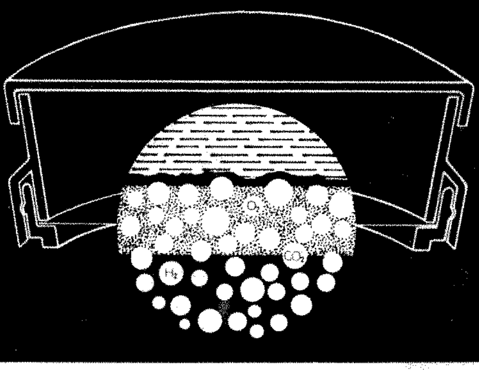
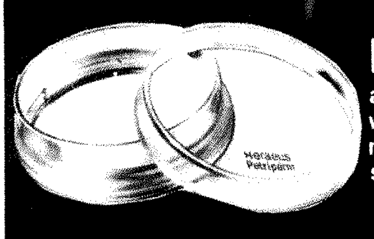
A new approach in tissue culture





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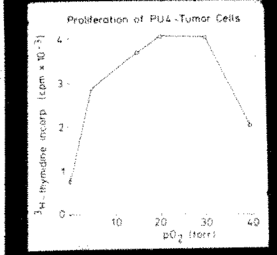

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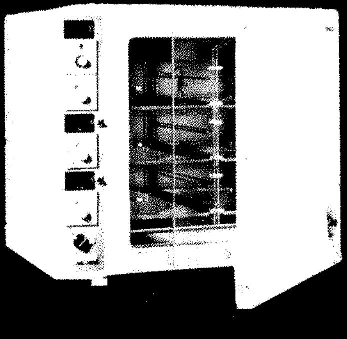

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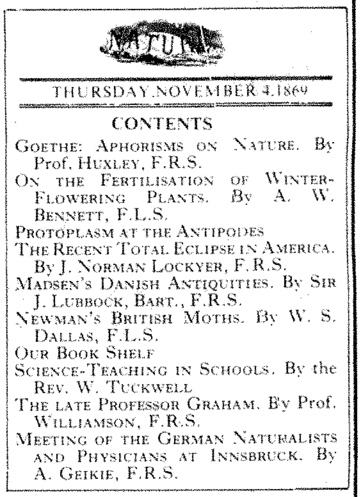

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Spanish success story

Workers' co-ops have taken root in the Basque country; could they in Britain?

Few would disagree these days that large parts of British industry are in serious decline, and that in some cases this decline may be irreversible. Almost everything has, at one time or another, been blamed for this, but there is little doubt that one crippling aspect in many industries is the immense distrust that exists between management and the workforce. Indeed, many of those involved in the pitched battles which occupy the headlines day after day speak as if conflict is a necessary ingredient in industrial relations. Those driven to despair by arguments over the sizes of the slices being cut from a rapidly shrinking cake may be interested to know that a totally different form of industrial structure has been quietly constructed in the Basque provinces of Spain in the last twenty years; and it shows signs of considerable health. It is the workers' co-operative movement centred on Mondragon, about 450 kilometres from Bilbao.

Basque Spain is a surprisingly industralised area. There are numerous towns and villages with all manner of industry from the lightest to the heaviest, much of it blithely producing extraordinarily high levels of environmental pollution. A Basque priest, José Maria Arizmendi, came to Mondragon, an economically devastated and repressed town in the midst of this industrial area, in 1941. His first job was to try to improve secondary and technical education which was at that time minimal. As educational standards gradually rose, he and some of his first students started to ask whether they could not participate more directly in industrial enterprises than merely as employees of capitalist firms. Out of these discussions came the first workers' co-operative, ULGOR, in 1956. A manufacturer of domestic appliances, it began with 23 people; it now employs around 3,500. In the past twenty years, more than sixty co-operatives have been founded, and they employ around 15,000 people. They range from agricultural co-ops through to iron and steel foundries. The unifying feature is that no employee can join without taking a stake (of between £1,000 and £2,000) in the enterprise. Basques are traditionally savers and there is said to be no shortage of applicants.

Although management of the co-ops is ultimately in the hands of the whole work force, care is taken to give managers many of the freedoms of their cpitalist counterparts to run their co-ops imaginatively. They do not, however, have the freedom to sack employees. Salary levels for each job are publicly established, and there is only a ratio of 3:1 between the top and bottom salary rates. Those retiring from co-ops now are receiving at least £10,000 in addition to their pension.

It is not just the workers who co-operate — co-operatives themselves all work closely together. The most striking example is the communally owned bank, Caja Laboral Popular, which acts both as a conventional bank for the workers and as a very sophisticated merchant bank for the support of existing co-ops and the establishment of new ones. Other examples of so-called second-degree co-operatives include schools, a social security organisation and a research and development centre. There is no doubt that most of the co-operatives have been successful in conventinal economic terms, in addition to providing an apparently happy working environment in which there is an openness in all acitivities. Managers speak enthusiastically of the common purpose in their factories, the willingness to work all hours to get satisfied customers and the high level of productivity.

Could Mondragon-type co-operatives survive in Britain? In the past few years the handful of older co-ops in Britain, many with structures very unlike those at Mondragon, have been joined, with maximum publicity, by co-ops encouraged by Mr Tony Benn. The trouble is that they have only become co-operatives as a last, desperate resort when a capitalist venture has failed. And indeed one, the *Scottish Daily News*, soon folded. The Mondragon experience points quite unambiguously to the importance of local workers coming together, probably on a small scale to start with, with their own ideas for something entirely new. □

- Two valuable books on worker co-ops are: *Worker-Owners* (Anglo-German Foundation, St Stephen's House, Victoria Embankment, London SW1; £2.90) and *The Case for Workers' Co-ops*, by Robert Oakeshott (Routledge & Kegan Paul; £7.95).

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Shuttle problems delay next planetary mission

David Dickson reports from Washington on why Jupiter must wait

The US National Aeronautics and Space Administration announced last week that it intends to delay the launch of Project Galileo — a two-vehicle mission to the planet Jupiter — from 1982 to 1984 because of problems in the development of the space shuttle.

The two-year delay will also mean separate launches for the two vehicles, one of which will orbit the planet, and the other descend into its atmosphere; initially plans were to use a single launch, but the delay means that the spacecraft will no longer be able to make use of extra push given by the planet Mars.

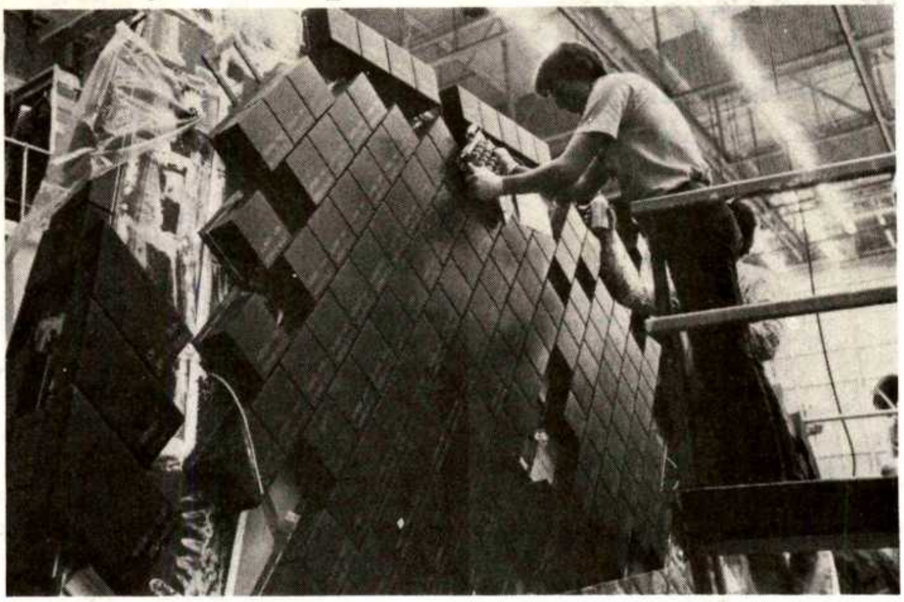
Dr Thomas Mutch, associate administrator for space science, told a House subcommittee in Washington last week that the agency had studied the implications of delays in developing the shuttle engines and decided that the risks involved in meeting the necessary schedules for both the shuttle and the inertial upper stage from which Galileo will be launched were "unacceptable". "The most cost-effective and least-risk approach to retain the Galileo scientific objectives is to delay the launch until 1984, and launch the orbiter and the probe separately", Dr Mutch said.

The delay, and the need for a double launch, will mean a considerable increase in the cost of the project. Originally, with a 1982 launch, this was estimated at about $450 million; the new estimate, including in particular the extra hardware which the double launch will require, is $675 million.

Dr Mutch told *Nature* that the new plans, which will involve launching the orbiter several weeks ahead of the probe, will not effect the scientific programmes, and could indeed increase the quality of the results. "Since there will be a separate carrier devoted to the probe, we can now optimise its trajectory to get the maximum coordination with the orbiter," Dr Mutch said.

However the delay could mean further problems for the Galileo programme from Congress, which will have to give its approval to the new schedule and the increased cost. Two years ago, a bid to kill the project by Representative Edward Boland, chairman of NASA's appropriations subcommittee, was defeated on the floor of the House, only after a massive letter-writing campaign by the academic community.

This year, given the delays with the launch, the House accepted an amendment to NASA's appropriations bill proposed by Mr Boland which would give the subcommittee power to veto any move to split the mission. This has been rejected by the Senate, however, and negotiations are now taking place to find an adequate wording to meet the House's concern for a review of the new plans, but without giving either legislative body veto powers.



Sticky problem for the space shuttle: some of these protective tiles came off in flight

NASA scientists are worried that Congress may try to reduce the costs by splitting the launch, and sending one half of the mission — possibly the orbiter — at a later date. They emphasise that NASA has no plans to split the mission in this way.

Meanwhile Dr Robert Frosch, administrator of NASA, told the House subcommittee that NASA calculated a 50-50 chance that the space shuttle would have its first flight by next summer. The flight was to have taken place this spring, but has been postponed after a series of technical problems. The most recent was replacing some of the thermal tiles, used to protect the re-usable shuttle when it re-enters the earth's orbit, which came unstuck during testing.

Admitting that this was a problem which "should have been clear sooner, but was not," Dr Frosch told the subcommittee that about 7% of the tiles so far tested had had to be replaced, implying a total of 2,400 out of the shuttle's 34,000 tiles.

Dr Frosch said that technical delays, in particular with the testing of engine performance, meant that the total cost of the shuttle development programme was now likely to be about 20% more than the initial estimate of $5.15 billion at 1971 prices. He told the subcommittee that NASA had made a number of management changes after investigations to discover why the shuttle programme had run into problems at this stage. But he emphasised that a major factor had been serious underfunding of the programme early on. □

IQ tests 'unconstitutional'

In what promises to be a landmark decision after a seven-year court battle, San Francisco's chief district court judge, Robert Peckham, has ruled that the use of standardised IQ tests to categorise children as mentally retarded discriminates against minorities and is thus unconstitutional.

The immediate effect will be to make permanent a state-wide injunction against these tests and to force state officials to re-evaluate black children previously identified as "educable mentally retarded".

The judge struck out at what he called the "grossly disproportionate enrolments of black children in the so-called 'educable mentally retarded' classes". Although black children make up only 9% of the state school population, some 23% of the children enrolled in these classes are black. Not only is this unconstitutional, Judge Peckham ruled, but it violates federal civil rights laws and statutes dealing with education for the handicapped as well. He ordered state officials to "monitor and eliminate" the disproportionate placements. The complex case was launched in 1971 by civil rights groups acting on behalf of six black children in San Francisco — known only as Larry *et al*. The children had been placed in special classes because they had scored lower than 75 on IQ tests. When they were tested later by a group of black psychologists, their scores ranged from 17 to 38 points higher. State officials argued that the testing protocols and procedures used by the black psychologists violated accepted practice.

However, the case apparently was decided more on the basis of the general limitations of IQ tests rather than on the specific problems of these particular children. In testimony that ran for more than 10,000 pages, Peckham heard a wide

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range of arguments over the validity and proper use of IQ tests. Interestingly, neither side took a firm stand on the issue of whether or not "intelligence" as so measured represents an in-born ability. Rather, attorneys for the children argued that the tests are culturally biased, while the state argued that the tests remain good predictors of school performance for blacks as well as white.

The judge said that, in his opinion, "many black children have been isolated, stigmatised and provided with inadequate education on the basis of unwarranted and impermissible assumptions". The tests, he said, had not been modified or validated for blacks, while "almost no experts now contend that IQ measures innate intelligence".

Beyond the issue of the tests, themselves, Peckham found that the special classes are designed to provide only a "limited, dead-end education for children who, while not severely retarded, are incapable of mastering the skills taught in a regular curriculum". Since placement is assumed to be permanent, he said, "the children assigned to those classes are unlikely ever to succeed in school, even if they are not truly retarded."

Black pupils: tests discriminate against them

Just what the ultimate effect of this ruling may be remains unclear. Despite the injunction, enrolment of blacks in mentally retarded classes remains disproportionately high. IQ tests can still be used to identify gifted children and those that qualify for some other special programmes. An appeal may be launched and related cases are pending in other parts of the country.

The case brought into full public view many of the problems related to IQ testing that have been argued about in scientific circles for many years. And there may be a growing sentiment in favour of paying more attention to "performance" rather than "ability". In an editorial following Peckham's decision, the *San Jose Mercury* suggested: "It would be far better for Larry P, and for students of every race, if educators would drop IQ tests entirely and rely instead on empirical evidence. Put the Larry P's in a regular classroom and see if they can handle the work. The time to move them into special programmes is after they have demonstrated an inability to keep up with their classmates."

John Douglas

Dispute over Dow Chemicals' theory of dioxin traces

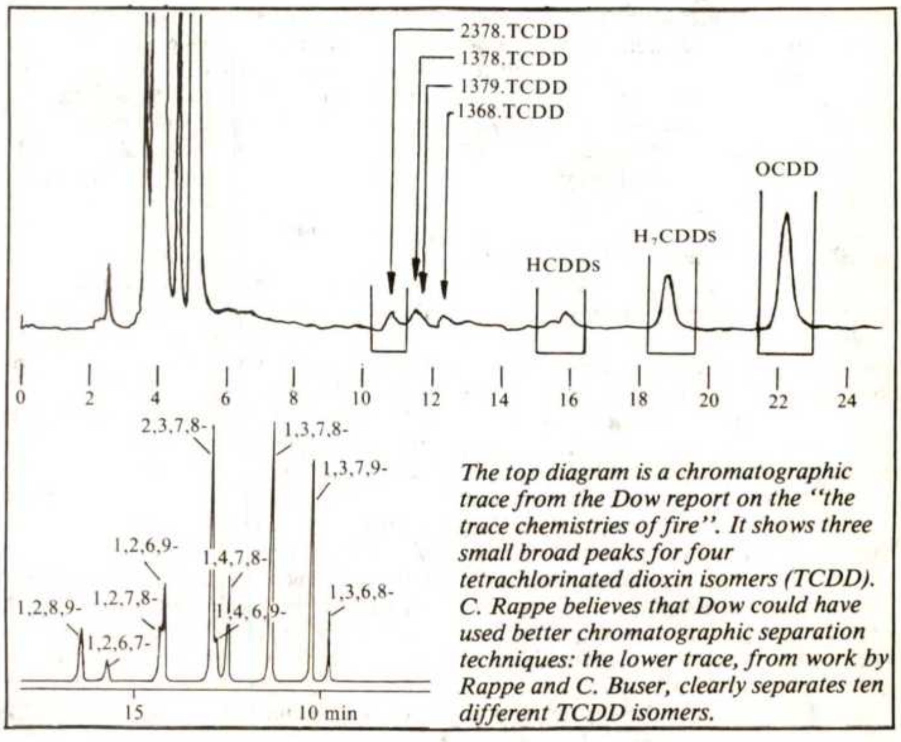
A Dow Chemical Company report on the toxic chlorinated dibenzodioxins, released in November 1978, has been severely criticised by Professor Christopher Rappe of the University of Umea, Sweden. He says that the methodology used by Dow is poor, rendering some of the results questionable; and furthermore, the conclusions — that dioxins are ubiquitous and a natural consequence of combustion processes — are far from proven. They are not borne out by results from Rappe's own laboratory.

The report, on "The trace chemistries of fire", was prepared for the State of Michigan's Department of Natural Resources (DNR), after Dow found that fish taken from the Tittabawassee River contained measurable amounts of chlorinated dioxins and polychlorinated biphenyls. Effluent from Dow's huge Michigan complex is discharged directly into the river and, not unnaturally, the company was assumed to be the source of the dioxins. Dow now dispute this charge: their report claims that dioxins are produced in many combustion processes, and are widespread. It has not convinced the DNR, however, which is insisting on additional measurements. Dr Robert Bumb, Director of Research at Dow's Michigan complex, believes that such additional tests would not only be expensive, but of little value; he says that other scientists are now confirming Dow's discovery.

Professor Rappe told *Nature*, however, that he disputes many of Dow's findings. Their discovery that dioxins are produced in commercial incinerators is not new, two independent European groups having reported earlier that the fly ash of municipal incinerators contains polychlorinated dibenzofurans. It was Dow's subsequent 'discovery' that chlorinated dioxins were present in the ash collected from other combustion processes which led them to develop their theory on the 'trace chemistries of fire'. Dow claim that dioxins are also present in ash collected from chemical tar-burners, fossil-fuelled power plants, the 'mufflers' of automobiles and trucks, household chimneys, cigarettes and even charcoal-broiled steaks. There had to be a common factor to explain the wide occurrence and Dow developed the theory of the trace chemistries of fire — defined as 'numerous chemical reactions occuring during combustion at very low concentrations, parts per million and lower'. Yields from these reactions are very low, of the order of 10^{-9} per cent.

The company attributes the formation of chlorinated dioxins to the presence of dioxin building blocks, which would include chlorine and chlorinated aliphatic and aromatic hydrocarbons. Metals present may act as catalysts "in a sea of chemical reactivity including pyrolysis, oxidation, reduction and acidloysis". In similar poetic vein, the report adds that in this sea, "ions, electrons, free radicals, free atoms and molecules form, combine and decompose". Chlorinated dioxins, Dow suggests, must be formed in this process.

Not necessarily, says Rappe. His results, those of colleague Dr Hans Rudolf Buser, and of Dr Hans Paul Bosshardt of



The top diagram is a chromatographic trace from the Dow report on the "the trace chemistries of fire". It shows three small broad peaks for four tetrachlorinated dioxin isomers (TCDD). C. Rappe believes that Dow could have used better chromatographic separation techniques: the lower trace, from work by Rappe and C. Buser, clearly separates ten different TCDD isomers.

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the Swiss Federal Research Station, all suggest that the dioxin precursors are not so nebulous. Rappe considers that chlorinated phenols and chlorinated diphenyl ethers are the main precursors in municipal incinerators. When these two groups of compounds are heated under laboratory conditions, Rappe has found that they produce isomers of chlorinated dioxins in similar proportions to those seen in commercial incinerators. Although Rappe forwarded his results to Dow, it appears either that they were not interested or that they chose to ignore his findings, for there are no analyses for these precursors in the Dow report.

The chromatographic separation techniques used by Dow to identify dioxin isomers are poor, according to Rappe, and their samples should be reanalysed using another method (see diagram on p619). In Rappe's opinion, Dow have overestimated the quantity of the dioxin isomer 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) which would be present in the fly ash of municipal incinerators. This isomer is many times more toxic than any of the other chlorinated dioxin isomers studied so far, and Rappe says that it represents only 1-3% of the tetrachlorinated dioxin isomers in European incinerator fly ash. Dow, on the other hand, report that in some cases 2,3,7,8-TCDD was the major tetrachlorinated isomer. Rappe suggests that this dramatic difference between his and Dow's results could be explained by the fact that Dow analysed samples from one of their own incinerators. Dow incinerates residue from the reactor used to make 2,4,5-trichlorophenol, the precursor of the herbicide 2,4,5-T. As the incineration of 2,4,5- trichlorophenol is known to produce 2,3,7,8-TCDD, Rappe suggests that the chlorinated phenol may have already been present in the reactor.

Rappe and his colleagues have so far prepared and identified 31 of the 75 chlorinated dioxin isomers which are considered to be theoretically possible. Although Dow used a far fewer (and unstated) number in its study, they believe that they identified enough isomers to make the report valid. In addition, Bumb points out that another Dow scientist, Dr D E Townsend has verified the trace chemistries theory from thermodynamic principles. Bumb told Nature that Townsend, in an unpublished report, finds a striking correlation between observed and predicted values for the dioxin isomers. Rappe disputes one of Townsend's assumptions (a constant ratio between dioxin isomers with different numbers of chlorine atoms): "consequently his basic theory is wrong".

Dow appears to be unaffected by the scepticism of scientists in Europe and the US. Bumb claims that "results which depart from traditional and commonly-held beliefs routinely provoke scepticism". And he remains confident in Dow's results and the company's conclusions.

Bumb and Rappe will have a further opportunity to put their respective cases in November, when both will present evidence in hearings organised by the US Environmental Protection Agency. The EPA is to consider its ban on the herbicide 2,4,5-T, which it has recently extended, from a restriction to forestry only, to a total ban covering all known uses of the herbicide in the US. The danger of 2,4,5-T lies in its contamination with small amounts of the dioxin 2,3,7,8-TCDD, one of the subjects of contention in the Dow report.

Alastair Hay

Israeli universities face financial crisis

ISRAEL'S seven institutions of higher learning have successfully resisted a recent attempt by the Knesset's Finance Committee to cut £8 million from their budgets.

A threat to double tuition fees or even close down campuses was enough to spur Prime Minister Menahem Begin to intervene. He successfully asked the committee to reverse its decision.

University officials are naturally relieved, but they are still fearful of future developments. Their mood was expressed by a top academic administrator who said that they "had won the battle and yet might go on to lose the war".

This is because the Knesset members who reluctantly agreed to restore the £8 million and many other influential Israelis apparently believe that the universities and academic research centres are characterised by luxury and waste, which means that they can more readily absorb budget cuts than can other institutions supported by public funds. And such cuts are inevitable if the rampaging, nearly three-digit inflation now plaguing Israel is to be brought under control.

The institutions of higher learning certainly helped to create their image in what Weizmann Institute President Michael Sela some years ago called "the Herodian era of construction", when universities, egged on by donors anxious to have their names associated with imposing edifices, seemed intent on surpassing one another in square metres of marble and concrete.

Yet there are also other, perhaps more significant factors at work. To some extent, as in the West generally, science and scientific institutions are held responsible in Israel for almost all the ills of modern society. In addition, parliamentarians concerned about the problems of the underprivileged and poorly educated sections of Israeli society are ready to divert scarce funds from universities and research centres to nursery schools, primary schools and secondary schools.

In any case, even before their latest tangle with the Knesset's Finance Committee, local institutions of higher learning were already suffering from a severe decline in government support. In the period between 1972 and 1978, when the national budget grew in real terms by 30%, funds allocated to higher education declined in real terms by 20%.

Making things even more difficult for the institutions is the erratic manner in which government allocations are dispensed. Money does not come in regularly week by week or month by month; instead most of it tends to arrive towards the end of the fiscal year.

Yet the universities are prohibited by law from withholding wages, and if they don't pay for their supplies, the supplies stop. This forces them to take high interest loans in order to bridge the gap between current expenditure and eventual government grants. University spokesmen claim that economy measures have already gone past the stage where fat was being trimmed and now are impairing their ability to operate properly. According to Tel Aviv University President Haim Ben-Shahar, "libraries and laboratories are no longer up to date. Journals containing important current research developments are sometimes impossible to acquire. Our scientists are forced to work with obsolete equipment, which, of course, puts them at a disadvantage in comparison to their colleagues abroad".

A research chemist at another institution told of a case in point. For some years, he said, the people in his department have been seeking funds to purchase a gas chromatograph mass spectrometer. At first they could go on with experiments by constantly tinkering with their old mass spectrometer, purchased in the early 1960s. But now some lines of research have had to be dropped as it is impossible to obtain relevant and significant results without the newer instrument.

Financial problems have severely limited the hiring of new staff, leaving institutions of higher learning with a disproportionate number of aging, tenured men and women. This news about job prospects has reached university students, and it undoubtedly has something to do with declining enrolments in the natural sciences.

Chemistry faculties are particularly hard hit, with registration down by anywhere from a quarter to three-quarters and some institutions now boasting more teaching staff than students. The same applies to a lesser extent to other science faculties. Students are now choosing short courses promising good employment prospects and the possibility of high financial gain.

Nechemia Meyers

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Public spending cuts threaten UK universities

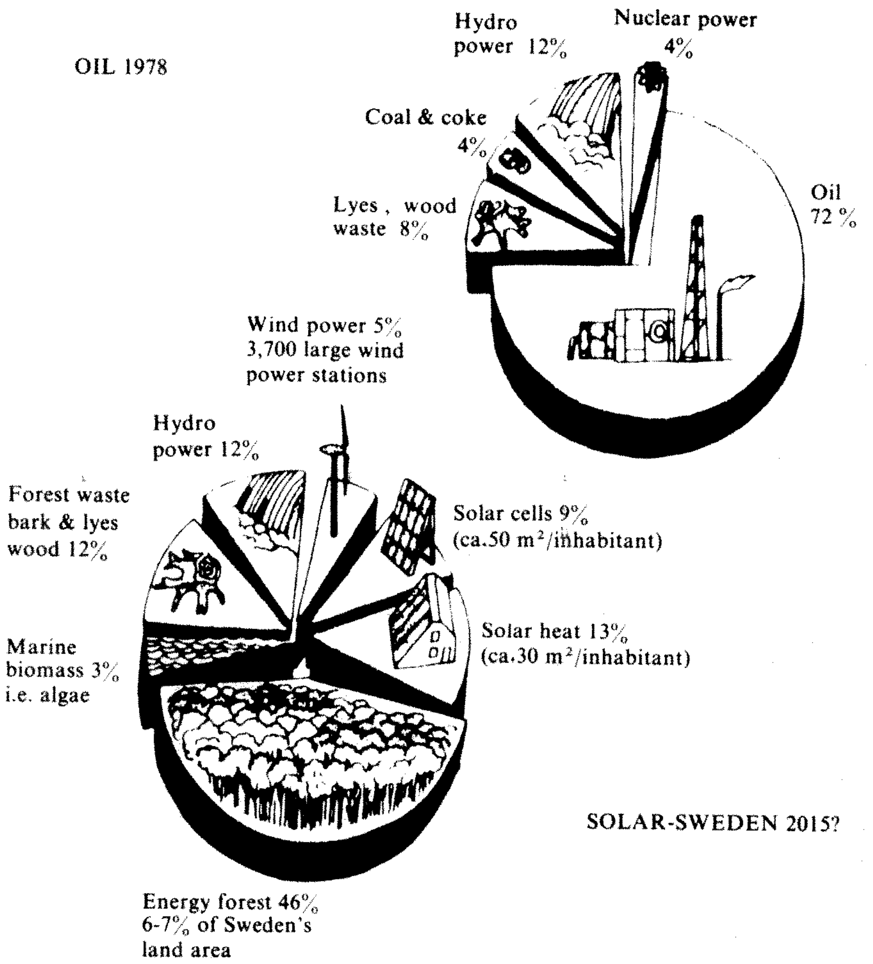
THE UK government is on the brink of a decision which could be disastrous for the country's universities, according to Sir Alec Merrison, Chairman of the Committee of Vice-Chancellors and Principals. Proposed cuts in funds would almost certainly mean the closure of some departments and even whole universities, he told a press conference last week, and universities would not be able to educate well-qualified home students whose numbers are likely to continue increasing over the next few years.

The CVCP was responding to the government's intention — outlined in a letter from the University Grants Committee to all universities last week — to hold the universities' recurrent grant at the 1979-80 level for several years and remove all UK government support for overseas students embarking on their studies on or after the 1980-81 academic year.

Cutting out funds for overseas students would result in a 13% fall in university income by 1983-84. The money could only be recovered if large numbers of overseas students were capable of paying their own fee — estimated at £3,500 each. This is highly unlikely, says the CVCP in a letter to Mark Carlisle, the Education Secretary. Overseas students would have to pay many times more to study in the UK than in other western countries and would therefore go elsewhere. British universities would cease to compete internationally. Some universities would be hit very badly, for example, the University of Manchester Institute of Science and Technology, a third of whose students come from overseas.

The vice-chancellors are concerned that research as well as teaching is bound to suffer. In looking for economies, universities would tend first to cut spending on research equipment. "The whole question of research is considerably at risk", Sir Alec said.

A further problem for universities is uncertainty about the 1979-80 level of funding which is to be used as the base for the recurrent grant until 1983-84. Salaries for university staff have yet to be agreed for the year ending July 1979 and the government has given no assurance that it will meet any increases. The CVCP is also concerned that pegging their future funds at 1979-80 levels will not take account of inflation and pay increases to come in the next few years. Many universities are already having difficulties existing on their funds. Sir Alec's own university, Bristol, ran up a deficit of £500,000 in an income of £25 million last year. "I'd be very surprised", he said "if there aren't some institutions that have had to go to the bank already." ☐



Changing the energy equation: how Sweden's forecasters see the future

Sweden warms to biomass power

INDEPENDENCE from oil as a source of energy was the basis of a Swedish contribution to a seminar on Technological Forecasting Methodologies recently held by the UN Economic Commission for Europe (ECE). The authors Thomas B Johansson of Lund University, and Peter Steen of the Swedish Research Institute for National Defence, suggest that 61% of the nation's energy could be provided by biomass. Six to seven percent of the country's land area, they propose, would be devoted to energy forests, estimated to yield 46% of the country's needs; forest waste and wood lyes (pulp effluent) would contribute 12% and marine biomass 3%. Other contributors would be solar heat (13%), conversion through solar cells (9%), wind, and hydro-power (12% — the same as the current figure).

No mention however is made of methane from the conversion of urban waste, a resource already used in some other north European countries. The figures are contrasted with those of the present system, which relies on oil for 72% of the country's needs, hydropower for 12%, woodwaste 8%, coal and coke and nuclear 4%. Neither coke nor nuclear appears in the 2015 forecast, which is said to allow for "moderately increased efficiency" and "twice the production of goods and services compared with today".

The authors have assumed that "the energy system should be fully implemented in the year 2015", and have calculated backwards the necessary rate of expansion. This gives 50% implementation from renewable resources by the year 2000. The rate they estimate is less than that estimated by the Swedish National Board for Energy Source Development. At the same time the cost of the proposed system is "not in conflict with production levels assumed for the whole economy" while it is based on a continued increase in labour productivity.

But there are difficulties. For example, there are to be major political and economic issues with regard to land-use policy and the capital market, since the proposals would require more land area and are more capital extensive than the present energy system. However, Sweden is already very advanced in research on energy plantations, making use of selected strains of various tree species grown on a short rotation system and giving yields comparable to those found in the tropics.

Two German papers at the seminar, however, estimated that solar energy would contribute only 5% of all energy by the year 2000. They paid little attention to the indirect uses of solar energy through biomass conversion or the better use of wood and wood-products. However, several other ECE meetings have been

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devoted to the energy aspects of the forest industries. At one it was indicated that the energy input of the industry itself to pulp and paper manufacture ranged from 5-35% in different countries, with a very much lower figure (5% only) for the input to the mechanical processing side. Sweden, again, is a leader in examining how these figures could be improved by more efficient use of forest waste: one recent estimate gives 50 million m^3 of residues consumable as fuel if not partially recovered for other processing, for every 60 million m^3 of commercial round wood produced annually.

Again, in Finland it is estimated that small sized wood, stump/root systems and other forest waste could provide 3.6 million tons oil equivalent a year, with another 0.8 mtoe depending on the market for certain hardwood products. Timber industries, faced with rapidly rising energy costs from conventional sources, are examining new ways of using their own waste products, either by direct combustion or through conversion into gas or liquid fuels. An advantage is that wood is not carcinogenic and is low in both nitrogen and sulphur. According to one ECE reort, forest product industries can and should become energy independent soon, later supplying surplus biomass to other users.

Peter Collins

Poland calls for wider research cooperation . . .

International cooperation at every level — from pure academic research to industrial design and implementation — is essential if a country's research potential is to develop to its full capacity. So Jan Mazurkiewicz, Director of 'Export and Economic Relations with Abroad' of the Polish Ministry of the Machine Industry told *Nature* recently. He was summarising the significance of "Poland's Technology, 1979" a series of seminars and technical exhibitions held last week in London, Manchester, Birmingham, and Coventry.

The close integration of research with industry is a basic feature of Polish planning. It was perhaps less familiar to some of the British participants, who seemed a little at a loss as to whether to expect learned discourses or sales promotion talks. For the event, sponsored on the British side by the Chambers of Commerce of the host cities, was the reciprocal or a similar British week in Warsaw last autumn, and represents one of the more positive results of Poland's large trade gap with Britain.

If trade between the two countries is to increase — and the state of the Polish economy makes this highly desirable — some new form of cooperation must be found. Recently, joint research and development projects, culminating in joint production for a third market, has increasingly been proposed on both sides as an attractive solution.

Any viable proposal for joint projects presupposes, that both sides know what the other has to offer. "A symposium means the meeting of people with like interests", Mr Mazurkiewicz explained. "So we chose our team very carefully — a couple of academicians, a test pilot, two plant engineers, an administrator, and so on. We looked for a good variety, for people with open minds, with whom there could be a point of contact."

The subjects, which ranged from medical electronics to machine tools and from computer software to agricultural aviation, were selected by the Polish side as fields where they felt they had something valuable to offer. Inevitably, in the discussion of any cooperation projects, sooner or later the problem of strategic military embargoes crept in. These, however, were peripheral, and in one instance, Jacek Szporko of "Unitra-Elektron" joked that the strategic embargo of the early 1960s had indirectly led to Poland's present expertise in semiconductor technology.

The close links between Polish research and industry meant that the Polish team could include lecturers of considerable international standing. Andrzej Radziminski (software and computer services) is a member of the Advisory Committee for Informatics Projects of the International Bureau for Informatics Dr Tadeusz Zak, who lectured on the Polish technological equipment industry, is a member of the Comecon Plenipotentiary Committee for Corrosion Problems, and Dr Halina Leszcynska (industrial sulphur processing) was closely involved in the development of granulated sulphur. This is free of the health and environmental hazards that had been closing more and more ports throughout the world to sulphur in its traditional powder form.

Dr Leszczynska is a member of the Committee of Chemical Sciences of the Polish Academy of Sciences, and, as such, represented a field which, she said, is always in the "top three places of research investment", due to its "importance in the national economy". She was particularly optimistic about the outcome of the week. "There are already a lot of people doing joint research at post-graduate level", she said. "What this week has done is to provide new direct contacts at the development level."

Several members of the Polish team, in informal discussions, suggested that joint industrial and development projects should ideally be based on truly joint research, rather than bringing together the results of research at a late stage.

Although the symposium, was slanted rather towards the industrial end of the research and development strectrum, the lectures from time to time revealed interesting sidelights on Polish research structure which would undoubtedly have to be taken into account in the planning of any joint research. Who would have guessed — until test pilot Wieslaw Mercik pointed it out — that the Warsaw Aviation Institute does no work at all on whole-body helicopter design, but is simply responsible for testing the aerodynamic and performance characteristics of designs produced in the factory design offices?

Vera Rich

. . . while the East Germans must wait for a trade agreement first

THE German Democratic Republic, which celebrated its 30th anniversary recently, is a country where, according to Dr Lutz Buschendorf, First Secretary (Science and Technology) of the GDR Embassy in London, every single worker is to some extent involved in science. Unfortunately, he said, so far scientific cooperation between the UK and the GDR has not developed to the extent one might have hoped. The reason, from the East German point of view, is the "unwillingness" of the UK to sign a cooperation agreement in which science and technology would not be linked to trade.

At present, technological cooperation comes under the Joint Agreement, originally drawn up in 1973, in which the British side comes under the aegis of the Department of Trade. This is the standard situation for all UK cooperation deals with Eastern Europe. The involvement with trade has a two-fold basis. Firstly, from the beginning, the UK felt that if the agreements were to bring genuinely mutual benefits, then some kind of counter-trade would be needed to off-set a predominantly one way, West to East flow of technology. Secondly, the administration of the agreements by the Department of Trade (via a special technology unit) is a result of the way the ministry of technology was dismantled.

To the East Germans, however, the link with trade constitutes a major psychological block. When the then UK Secretary of State for Trade, Edmund Dell, visited the GDR some three years ago, he stressed that under existing economic conditions the trade turn-over between the UK and the GDR could well be doubled. Some Germans seem to have taken this as a direct challenge to their thesis that increased scientific and technological cooperation should pre-date any major expansion of trade. "We want to make

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cooperation more long-term", Dr Buschendorf explained. "Under the present agreement it is difficult to get the right partners for scientific cooperation".

From the German point of view, the situation was somewhat improved by the signing, last May, of an agreement for the exchange of scientists, between the Royal Society and the GDR Academy of Sciences. Otherwise, however, they remain intransigent that what they need is a separate cooperation for science. They do not even seem willing to discuss possible areas of cooperation until such an agreement is signed — even to suggest subjects, Dr Buschendorf felt, would be "premature". It is known, however, that the British side has put forward a number of concrete proposals for joint seminars and visits, in order to bring together specialists who could then discuss feasible cooperation programmes. Subjects in which Germans have indicated particular interest include industrial hydraulics and corrosion protection. So far, however, there has been no firm response.

The insistence of the GDR on keeping science and trade firmly separated seems a little incongruous in the light of its own science policy. This, according to the official hand-outs for the current anniversary, is based on "a close and desired connection" between research and "the tasks set in the national economy". Innovation and implementation are the keynotes, and workers at all levels are encouraged to suggest further possible improvements. There are close funding links between industry and basic research. The production of university graduates is also a part of state planning, and each student is provided with his or her future job at least a year before graduation.

Given an economic structure, where "all the planning of scientific and technological work is based on the requirements put on the development of the national economy", it is difficult to understand the Germans' reluctance at least to explore what can be done under the existing agreement. Many of the fields which have priority in the GDR research programme — environment, water pollution, energy and raw materials, computers, could provide considerable scope for joint work with the UK or other western countries.

Perhaps some part of the explanation may lie in a report produced last spring by the East Berlin Institute for Politics and Economics. The Institute team, it appears, was apprehensive that injudicious importing of licenses could harm indigenous research and development by turning the national research potential into a mere mechanism for the adaptation of foreign thinking.

So far, however, no negotiators have made any reference to this point of view, but, on the thirtieth anniversary of GDR statehood, have raised yet again their plea for a separate scientific agreement.

Vera Rich

Soviet scientist proposes Siberian sites for nuclear power stations

Soviet nuclear power stations should be sited in remote regions of Siberia and the Far North, according to Academician Nikolai Dollezhal' a leading nuclear scientist, and Yurii Koryakin, a Doctor of Economic Sciences. Moreover, such power stations should take the form of large "nuclear energy complexes", each consisting of a number of power stations of "several tens of millions of kW capacity" together with fuel reprocessing plants, and possibly facilities for utilising nuclear byproducts.

This proposal, which the authors themselves admit is "radical", appeared in the September issue of *Kommunist*, the leading Party theoretical journal. Starting out apparently as an encomium of Soviet nuclear progress, the tone gradually becomes less optimistic, and, after outlining the setbacks and delays of the fast breeder programme, the authors openly tackle the environmental problems, hitherto dismissed by Soviet publicists as scare-mongering by capitalist oil interests. Reprocessing risks are particularly stresed, and bolstered by the assertion that basic reprocessing costs are "significantly" higher than originally estimated.

Still more costly, they imply, is the loss in the productive land and water resources. Some 60% of the Soviet population live west of the Volga river/Volga-Baltic canal line. It is here, in the main area of industrial and agricultural production that it is currently proposed to site nuclear power stations. The annual water requirements of nuclear and thermal power-stations already exceed 100 km^3, some 2 km^3 of which are "irreversibly" lost. To accommodate this water, by the end of the century, if current construction patterns continue, agricultural land sufficient to provide bread for "several million people" will have been sacrificed.

Dollezhal' and Koryakin would not, they stress, cast any doubt on the "historical necessity" of nuclear energy. Without nuclear energy, they affirm "it is impossible to construct the energy basis of developed socialism".

Giant energy complexes in Siberia, they claim, might well prove to be more efficient as regards construction, operation, and the "utilisation of labour reserves". Environmental hazards would be removed to a safe distance, and those associated with the transport of fuel from reactor to reprocessing plant eliminated completely. Finally — and this might well be the point that would win over the decision makers — the proposed nuclear complexes could be integrated into the Soviet Union's most prestigious civil engineering project — the diversion of the Siberian rivers to replenish the arid south.

Vera Rich

Physicist flown home to face charges

Franco Piperno, the Italian physicist who had asked for political asylum in France, was extradited to Italy last week. The extradition order was given a week in advance of its scheduled hearing to avoid demonstrations and rescue attempts.

Piperno, aged 36, was escorted from Sante prison at 7am to Le Bourget Airport where an Italian Air force plane took him to the small military airfield, Practica di Mare, north of Rome. He was whisked to solitary confinement in the political wing of the modern Revibbia jail in Rome.

Students in Rome set fire to cars and buses to protest at Pierno's extradition from France and police fired volleys of tear gas in unsuccessful attempts to disperse the demonstrators.

Forty five counts of extradition ranging from stealing car license plates to armed insurrection and including offences against the highway code were rejected by the French Court of Appeal (as was a first extradition request) on the grounds that they were political in character and violated the 1870 extradition treaty between the two countries.

But the court released Piperno to the Italian authorities to answer charges that he had sheltered two men in whose homes Italian police had found guns allegedly used in the abduction of Aldo Moro. Moro, one of Italy's political elder statesmen, was kidnapped and later killed by the Red Brigade guerrilla group in Rome after their attempts to exchange him for jailed comrades had failed.

Piperno had denied involvement in the Moro affair. His statement at the extradition hearing was: "Perhaps it is better this way. At least I will be able to confront quickly that which in Italy is called justice and to demonstrate that I was not involved in the Moro kidnapping and assasination."

In Paris, where Piperno had received widespread support, French commenators expressed dismay and anger at the extradition. Francois Mitterand, leader of the Socialist opposition, declared that the Socialist Party "deplores the fact that French judges have yielded to governmental pressures". And Phillipe Boucher, writing in *Le Monde*, said: "It is plainly unreasonable to believe that Italy had no political interest in brandishing Piperno as proof of their determination to combat terrorism. No one can seriously maintain the contrary . . . The (judicial) method in this case is contestable, indeed unseemly. It is furthermore dangerous." ☐

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Who shall inherit the seeds of the earth?

"Seeds of the Earth", the study recently released by the London-based International Coalition for Development Action (ICDA), raised several issues of international concern. The study's author P R Mooney predicts that the Green Revolution is now moving into its second phase — a dangerous phase in which the responsibility of plant breeding is largely being left to the large private multinational companies. The study contends that new legislation in the developed countries which gives patent-equivalent protection to producers of new varieties of plants is helping the multinational corporations to acquire dominance over the seed market.

These trends are dangerous, according to the ICDA study, because of the gradual erosion of the world's plant genetic resources that is taking place all across the Third World. This erosion process has been accelerated in the last decade by the Green Revolution which has helped to bring vast areas under cultivation of monocultures. Deforestation is another aggravating factor. The Vavilov centres of genetic diversity (see map) — the geographical regions from which plant breeders draw a large part of their germplasm — are mainly located in the Third World. This germplasm is vital to the future food supply systems of mankind, as it is needed by plant breeders for further improvement of crop yields and incorporation of other desirable characteristics such as disease and pest resistance.

'Seeds of the Earth' points out that private corporate interests which are entering the seeds business are building up private gene banks to which access is limited only to the companys' plant breeders. This situation is particularly ironical for Third World plant breeders as Third World countries are the original suppliers of these plant resources.

Little is known about private germplasm collections, but the study does claim that "in some crops a single enterprise dominates total world germplasm holdings". "The FAC reports", says the study, "that one company, United Brands (formerly United Fruit), has about two-thirds of the world's banana germplasm in storage". Some public gene banks have had difficulty in obtaining information from private companies about the quantity of type of genetic material they hold.

At the international level, germplasm conservation is the responsibility of the International Board for Plant Genetic Resources which is based in Rome within the Food and Agriculture Organisation of the United Nations. The board co-ordinates the regional germplasm collection work that is being undertaken by the eight existing international crop research stations. There are also some 60 nationally-controlled gene banks. The germplasm collection work is massive and the board's activities remain clearly underfunded. There is little germplasm collection work being undertaken by Third World governments themselves, whose plant heritage is immediately threatened.

The ICDA study, therefore, recommends the creation of an annual emergency budget of US $100 million for the collection and storage of genetic material and creation of biosphere reserves. But equally important, the study points out, is the step that the UN should take to declare plants as "resources of common heritage to all peoples". There should be no form of legislation that allows exclusive control over plants. Secondly, the study recommends that the genetic material collected should be stored in the developing country from where it is collected. Most of the germplasm being collected at the moment is stored in the developed countries. If this trend continues, the Third World, the original supplier of this vital resource, could soon find itself dependent on the developed countries for the basic material required for its own plant breeding programmes.

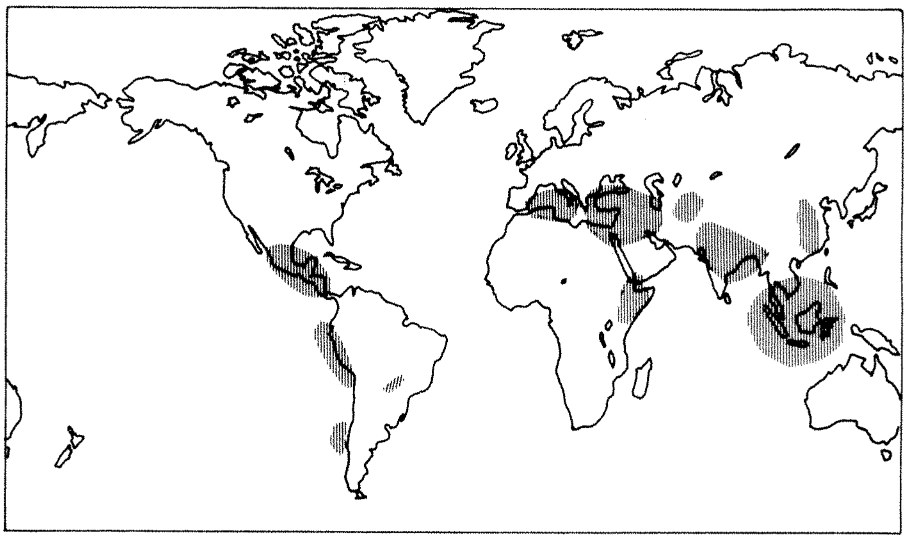
The immediate target of attack of the ICDA study is the new legislation that is pending in several developed countries. This legislation provides patent-like protection to new plant varieties. It is the opportunities provided by this type of legislation — often called legislation for "plant breeders' rights" — that has encouraged the entry of big corporate interests, much to the detriment of the small breeders who are now being squeezed out of the market or being bought up. The ICDA study, in fact, claims to have been born out of the movement that farmers in Saskatchewan province of Canada have launched against the pending Canadian bill on plant breeders' rights.

Unfortunately, the study fails to provide adequate evidence for some of the charges it makes. For instance, the argument that the entry of agrochemical companies into the seeds business is "a way of assuring that one product line needs the other" remains at the moment a mere hypothesis. Patent protection for many important pesticides that were developed in the 1960s and earlier has already expired or is about to expire. This has raised problems for many companies which are now taking various steps to protect their interests either by diversifying into related areas or by somehow ensuring that their sales are not affected by expiry of patents. As agrochemical companies already have considerable scientific and technical expertise in various branches of agricultural sciences and a good marketing network that reaches right out to the farmers' fields, the seeds business may just be the ideal area in which to diversify.

Similarly, the World Intellectual Property Organisation which is responsible for the International Union for the Protection of New Varieties of Plants (UPOV) believes that the fears raised about the effects of the PBR legislation are exaggerated. An article of the International Convention for the Protection of New Varieties of Plants clearly states that no authorisation is needed to use a protected variety to produce yet another variety — except in the case where "the repeated use of the new variety is necessary for the commercial production of another variety," as in the case of hybrid varieties. Thus, the PBR legislation should not in itself prove to be a major impediment for further plant breeding work.

But despite its deficiencies, there is no doubt that the ICDA study raises several issues of major concern to mankind, and these issues needed to be fully debated to protect public interest, both in the developed and the developing countries.

Anil Agarwal



The Vavilov centres of genetic diversity: the Mediterranean, the Near East, Afghanistan, Indo-Burma, Malaysia-Java, China, Guatemala-Mexico, the Peruvian Andes and Ethiopia.

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news in brief

Birmingham University smallpox case starts: Legal action against Birmingham University under Section 2 of the UK 1974 Health and Safety at Work Act began this week in Birmingham. The university is being prosecuted by the Health and Safety Executive for failing in its obligation to "ensure as far as reasonably practical" safe working conditions in the east wing of the University Hospital. The release of smallpox pathogens from a P3 containment facility late in 1978 resulted in the death of a laboratory worker and closure of the facility. Under the terms of the act, the university could face a maximum fine of £1,000.

UK experts in occupational health regard the case as an important precedent that goes beyond the guilt or innocence of the university. It is the first prosecution brought against a university since the act was passed in 1974. Sheila McKechnie, health and safety officer of the Association of Scientific, Technical, and Managerial Staffs, said the case had given impetus to the campaign for laboratory safety. "The universities have been hiding behind a lot of 'frontiers of science' rubbish. They have been arrogant and have refused to acknowledge their obligations under the 1974 Act. The smallpox case has raised everyone's awareness of the extent of health hazards involved in university research", she said.

US studies identify formaldehyde as carcinogenic: Laboratory rats exposed to formaldehyde vapour have been shown to develop nasal cancer, according to tests submitted to the Consumer Product Safety Commission by the Formaldehyde Institute recently. A spokesman for the institute said that in the course of a two-year study, four rats out of a total of 720 tested developed tumours visible to the naked eye after 16 months, three after exposure to formaldhyde exposure at levels of 15 parts per million, and one at a dose level of six parts per million. Under existing standards promulgated by the Occupational Safety and Health Administration, worker exposure must be kept below three parts per million over an eight-hour period, or an average of five parts per million over 15 minutes. Mr John Clary, chairman of the institute's medical committee and a toxicologist with Celanese Corporation, said that the results were "significant".

ARMS to lobby parliament: ARMS, the Association for Researchers in Medical Science, are holding a day of parliamentary activity on 6 November. Members and supporters will assemble outside the Department of Education and Science to present a petition to the Secretary of State for Education and Science demanding "immediate action to implement a proper career structure for research scientists in biomedical research in UK universities and medical schools". A meeting is then planned with a number of MPs to discuss the position of medical researchers in the UK. An ARMS spokesman called the action "perhaps the last chance for researchers on short term contracts to discuss with MPs what is wrong with the current system of funding in the UK".

Chinese Minister of Education in whirlwind tour of UK: Mr Jiang Nanxiang, Minister of Education of the People's Republic of China, visited the UK from 28 September to 7 October to discuss scientific exchanges and to see British educational institutions. Mr Jiang, who had been relieved of his duties as principal of Peking University during the Cultural Revolution, was appointed Minister of Education earlier this year. He is said to be a believer in "quality education". The one-week tour consisted of visits to 18 UK educational and scientific institutions including middle schools, polytechnics and the Clarendon and Cavendish laboratories at Oxford and Cambridge. The Chinese delegation expressed particular interest in the Open University and its sophisticated system of correspondence courses, television programmes and regional tutorial centres. According to an OU spokes-person, the minister expressed interest in the regional centres, home experiment kits, and asked whether OU degrees were useful for "advancement and promotion". The university is designed to make university education more readily available to people from working class backgrounds but the Chinese minister expressed "little interest" in this aspect of the university's work, an OU official said.

India achieves breakthrough in fish culture: India's fish production may be revolutionised by a new composite fish culture technique developed by the Central Inland Fisheries Institute, (CIFRI), Barrackpore, West Bengal. The new culture technique uses a compatible fish combination of indigenous major carps and fast growing carps, and scientists testing this found yields as high as 6,500 kg fish per hectare per annum could be produced in some regions, although the project target had been only 3,000 kg fish per hectare per annum. This culture can be integrated with pig and duck culture, reducing the operational costs and producing 4,500 kg fish per hectare per annum and 1,800 duck eggs. Pig and duck excreta supply nutrients to the water, maintaining a high level of production. CIFRI's director, Dr V G Jhingran, estimates that if all India's present culturable waters are put to composite fish culture, average fish production will rise from 600 to 3,000 kg fish per hectare per annum. — *Zaka Imam, New Delhi*

Most blindness can be prevented: A meeting of an international group of experts on blindness has produced a report claiming that the incidence of blindness in the world can be halved quickly by simple and effective preventive measures. According to the report, most of the estimated 30-40 million cases of blindness are due to infection, malnutrition and cataracts. Visual impairment is 10–40 times more prevalent in certain areas of developing countries than in industrialised countries. The main obstacles to initiating a prevention campaign are a lack of national commitment, inadequate administrative infrastructure and a lack of money. The report also urges an avoidance of "the misunderstanding that equates the prevention of blindness with the availability of opthalmologists". The meeting was sponsored by the F.I. Proctor Foundation in San Francisco, The International Eye Foundation, and the World Health Organisation. *Guidelines for Programmes for the Prevention of Blindness.* World Health Organisation, Geneva, 1979. 47pp. Sw Fr 10.

IAEA reports 50% increase in new nuclear plants: The International Atomic Energy Agency in Vienna, in its annual survey of nuclear energy, reports that 15,000MW of new nuclear plants were installed in 1978 compared to 10,000MW installed in 1977. Nuclear power now accounts for 110,000MW or 5.8% of the world's total electrical generating capacity with 227 nuclear power stations operating in 21 of the agency's member states. The agency defends the safety record of nuclear power, citing "twenty years of commercial nuclear power generation without a single radiation-induced death or a serious radiation induced injury at any nuclear power plant".

EEC to spend £70 million on energy research: The Council of the European Communities is to spend about £70 million on a second four-year programme into energy research and development. The money will be allocated on the basis of shared-cost contracts over the four-year period 1979–83. Research organisations, industrial companies and universities will be eligible to apply and the first formal call for applications has been made by the EEC. Applications will be screened by an advisory committee which will make recommendations to the commission. The breakdown of the allocation is: solar energy, £30.7m; energy conservation, £18m; geothermal energy, £12m; hydrogen energy production,£5.3m; and energy strategy studies, £4m.

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'There is a frustrating logical gap between the assertion that recombinant DNA poses potential risks . . . and the containment levels imposed by current guidelines'

Francis Rolleston proposes a formula to bridge the safety gap in recombinant DNA research and concludes that any approach which falls short calculation of the risks is necessarily misleading.

THE UK Genetic Manipulation Advisory Group (GMAG) has proposed an important new way to assess the relative possible risks of recombinant DNA experiments (9 November 1978, page 104), but it addresses only the assessment of risk, without establishing the necessary correlation between risk and the containment required to reduce the risk to acceptable levels. Here I will suggest a framework for discussion based on this essential correlation. This framework also offers a clarification of the roles of the scientists and the public in setting safety standards, and establishes a foundation for communication between them.

All current guidelines make quantitative statements; they are safety standards which state, in effect, that the inherent risks can be reduced to acceptable levels of public risk by the imposition of defined safety procedures. Yet these quantitative statements cannot be defended either quantitatively or logically. The guidelines therefore do not provide a foundation for rational interchange either among scientists themselves, or—more important—between scientists assessing risks and containment procedures and the public, who should be reviewing the assessments by the scientists and setting acceptable standards of public risk. There is a frustrating logical gap between the assertion that recombinant DNA poses potential risks on the one hand, and the containment levels that have been imposed by current guidelines on the other.

Logical guidelines must be both internally and externally consistent. Questions of internal consistency are illustrated by the option sometimes offered of a trade-off between physical and biological containment (do P2-EK1 and P1-EK2 offer similar containment?), or by the assignment of different experiments to different containment levels (are the same safety standards being achieved?). External consistency raises the question of whether the safety standards imposed for recombinant DNA are consistent with those required or accepted in other areas.

A conceptually simple framework that may meet these needs for consistency is offered by the formula for a straight line, $R = aC + T$. Here, R describes the risks inherent in cloning a certain gene in a certain host-vector system, C is the physical containment required, and t is a threshold level of acceptable public risk to which inherent risks (R) must be reduced, if necessary, by physical containment (C). In this general framework, the need for internal consistency is met if a, the slope, is equal to one. The value of T determines the external consistency of the containment requirements. The required distinction between the scientific and the public roles is achieved by recognising that the values of R and C are determined primarily in the scientific domain, whereas T should be set in the public domain. Both the numerical values and the explicit separation of the scientific and public roles in this equation allow—even force—the necessary interchange between scientists and the public.

In using this framework, the two variables R and C can be assessed in either an absolute or a relative sense. The absolute approach requires interrelated units in which to express R and C and therefore T. In the alternative approach, R and C do not have units, but are expressed relative to reference points; declaration of a relationship between the two reference points will define T, since the slope a must, for internal consistency, equal 1. The absolute approach is more satisfying, but probably can only be used in areas such as radiation damage or automobile travel where risks can be clearly identified and assessed; the relative appraoch appears preferable where risks are conjectural.

Such a relative approach to values for R was developed by Brenner and applied by Rigby, and their manuscripts formed the basis of the paper by GMAG. Their reference point was a very hazardous possible experiment in which the pure gene for cholera toxin is cloned and expressed in wild *E. coli*. The risks of other experiments relative to this reference were expressed in terms of three separate factors (damage, expression and access) which, multiplied together, gave values ranging from less than 10^{-16} to 1; these values were stated by the authors to be overestimates of relative risk.

The relative approach can also be applied to values of C. The reference point might be open to bench experimentation (P1 containment). With respect to researchers inside the laboratory, it can be suggested that a P2 laboratory offers perhaps 10^2 greater protection, allowing for the fact that the relative safety of a containment cabinet is limited by HEPA filter standards, and that not all procedures are carried out in such cabinets. A P3 laboratory offers perhaps a further protection of 10^2 since here airflow requirements reduce persistence of aerosols and more procedures are carried out in containment cabinets; and a P4 facility represents 10^6 greater protection than P1, again considering investigators inside the laboratory. Estimates for people outside the facility, or for the environment, result in smaller relative protection figures, since the only barrier is the HEPA filter, although the absolute hazards for those who work outside the facility are evidently much less than for those inside.

These primary figures illustrate how values for R and C might be developed by the scientific community. If the figures given have any significance the range of values for R is much greater than that for C. Therefore, the value assigned to T by the public process will determine whether some possible experiments are too hazardous to be performed at even the highest levels of physical containment, or alternatively other experiments present such low hazards that physical containment measures are superfluous; or perhaps both. If, for example, the reference experiment of cloning the

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The author is director of special programmes for the Medical Research Council of Canada, with responsibility for regulating recombinant DNA research.

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cholera toxin gene in a wild type *E. coli* requires P4 containment—stated by the Council of the Royal Society Report (*Nature*, 15 February, page 509) to be too severe a level for *Vibrio cholerae* — then any experiment with values for R less than 10^{-6} (the resulting value of T) can be performed at P1 or less containment. From the above figures, it appears that this would include most experiments now requiring higher levels of containment, and it would, in large measure, incorporate the conclusions reached at Falmouth, Ascot and Wye.

A second conclusion from these tentative ranges of R and C is that the value of a is considerably greater than one in the original American, British and Canadian guidelines (which tended to spread the range of experiments across the available physical containment levels) and even in the revised American and Canadian guidelines. Therefore, if it is claimed that the more hazardous experiments have been appropriately contained, then those with less risk have been over-contained; conversely, if the apparently less risky experiments have been set at suitable containment levels, then containment standards for those of greater hazard have not been strict enough. Extrapolation from current scientific opinion (as expressed at Wye, without as yet any voice to the contrary) indicates that, if one must choose between these two claims, the former would be more accurate.

This proposed framework rests on assigning numerical values to risks and containment. Such an approach has been criticised (15 February, page 509 and 8 March, page 113) as being too imprecise because the risks are conjectural and probabilities of human error cannot yet be assessed. However, the current approach to guidelines makes just such judgements without admitting to them. A formal framework for setting safety standards which also segregates the scientific and public roles, while providing a means of communication, may focus attention on specifics as opposed to generalities, and require those who make pronouncements of doom or optimism to define their statements more accurately. □

I thank Mark Pearson for a most useful discussion of the manuscript, and John Keyston whose comments in September 1977 on the need for interrelationships between risk and physical and biological containment I did not understand at the time.

MIT puts some polish on a tarnished image

The Massachusetts Institute of Technology has raised $4 million to establish an ambitious College of Science, Technology and Society. Supporters say it will help give MIT-trained scientists and engineers "an education for the 21st century"; others claim it is an attempt to protect private industry from criticism of its use of science. **David Dickson** reports

WHEN Dr Paul Gray spoke to the press earlier this month following his election as the next president of the Massachusetts Institute of Technology, he placed particular emphasis on the steps which the institute is taking in response to public criticism of the social impacts of science and technology.

The current centrepiece of this face-lift is an ambitious plan to create a new College for Science, Technology and Society at MIT. Already $4 million has been raised from outside to support the project; and a programme of research and undergraduate teaching has begun as a development phase, supported by a number of eminent faculty appointments (including the historian of science Thomas Kuhn).

There is nothing particularly unusual about a technological university starting a programme in this area. What distinguishes MIT's efforts, however, is partly the size and scope of its commitment; and partly the fact that the programme is less a direct product of concern about the social impact of science, than a second order "reaction to public reaction" about such impact, such as the growing debates over issues from nuclear safety to the carcinogenicity of food additives.

And this has provoked its own controversy. The architects of the course claim that it is "a way of matching the maturing desire of MIT to go in this direction with the maturing needs of the nation"; critics warn that the institute is being used to promote the strategies of private industry in politically charged debates over technological risks and the appropriate social response.

Despite outward appearances, MIT is no stranger to the humanities. Various moves have in the past been made to complement its more scientific and technical courses; the Lewis Report of 1949, for example, led to the setting up in the 1950s of both a school of humanities and social sciences, and the Sloan School of Management. More recently, there have been particular developments such as a Technology Studies Programme and a Technology and Culture Seminar.

The aim of the proposed college is to focus these various activities and give them a new prominence within the institute. The move stems largely from an initiative taken by Dr Jerome Wiesner, ex-science adviser to President Kennedy and current president of MIT, who set up a faculty task force in 1973 to advise on how these goals could best be achieved.

After consulting with numerous individuals inside and outside MIT, the task force recommended the setting up of an independent college, with its own faculty, research fellows and undergraduate teaching programme. Based on this report, the institute raised $2.5 million in 1977 from three foundations,the Sloan Foundation, the Mellon Foundation and the Flora Hewlett Foundation; a further $1 million was added this year from the Max Fleischmann Foundation for renovating an appropriate building, and a $500,000 grant for a fellowship programme was made by the Exxon Foundation.

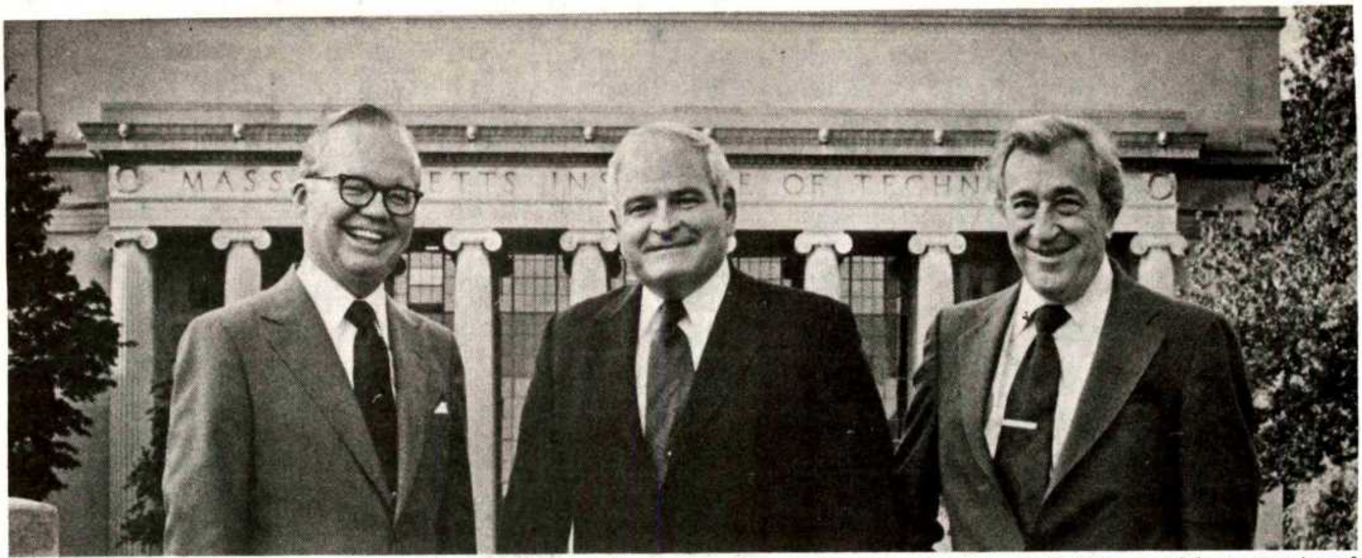
With this support under its belt, MIT has already been able to make seven new tenured appointments, and introduce a new undergraduate teaching programme — technically under the school of humanities — in areas ranging from the history of science and technology to "science, technology and the organisation of industrial society". A fellowship programme begins next year.

From the beginning, those responsible for the programme have emphasised that a high academic standard is necessary to avoid the 'credibility' problems associated with many previous attempts at multidiscipinary education. At the same time, however, it is stressed that the programme is also to be of direct use to the future scientist or engineer.

The 1977 prospectus for the college, for example, emphasises that the students will acquire "marketable knowledge", and that they will be "more effective professionals for this knowledge".

"The trends in terms of public perception of the implications of science and technology for society are continuing to mount, even if students are less vocal about it than they were at the beginning of the decade," says Dr Donald Blackmer, director of the new programme and deputy dean of the school of humanities. "The converse of this is that scientists and engineers are increasingly concerned about the impact of government and public attitudes on what they do."

This impact is felt both on the application of science, and on its practice. Dr Wiesner, for example, has been an outspoken critic of the extent to which social controls manifest as government regulation may be holding back both industrial innovation and university research. Addressing a meeting of the National Council of University Research Administrators in Washington earlier this year, he stated that "In the technical realm we can see many ways to continue humanity's forward march, if we do not



Behind MIT's new image: President Jerome Weisner (right) and his successor-to-be, Dr Paul Gray (centre). Left: chairman of the corporation of MIT, Howard W. Johnson

put too many shackles on those elements of society — universities, business, even some parts of government — that have made our technological achievements possible."

Closer to home, MIT biologists have had direct experience of the fierce and acrimonious debates that have surrounded the use of recombinant DNA techniques. Many were involved in public discussions over the extent to which the City of Cambridge should be permitted to exercise controls over such research.

Some now argue that it was a mistake ever to have raised publicly the potential hazards of this work. But Professor Gerald Holton of Harvard University, a visiting professor for the STS programme, argues that despite the "great cost and anguish", the debate was a "historic necessity", and that the main dangers lie not in attempts to restrict research as such, but the fact that they may be imposed from outside through an "uninformed bureaucracy".

"Scientists should not be pushed into a defensive position, when it has been the scientists who have historically dealt with self-imposed limitations, such as the discipline of the peer-review process. They should be aware that including these new issues is part of an on-going maturing of the profession" Professor Holton says.

Thus a general concern, not so much the need to control the uses of science as the institutional forms through which society has chosen and is likely to choose to exercise this control, forms a backdrop to the activities of MIT's new programme. In terms of study groups and lecture series, these range from topics such as technology and work, or risk and regulation, to environmental studies and the role of computers in society.

This concern is also directly linked to an issue which has caused considerable discussion within the programme faculty, namely the extent to which it should get explicitly involved in issues of public policy. Initially there was a certain reluctance to do so from anything more than a long-term, "basic research" perspective, a tendency illustrated by the high proportion of historians among initial faculty appointments.

More recently this emphasis has shifted, largely under promoting from Dr Wiesner, who stresses the "unique contribution" that universities can make to problems "that require mixed solutions that are at once technical, managerial, political and social." Dr Blackmer talks about bringing in more policy people as helping promote "a useful dialogue"; Professor Walter Rosenblith, MIT provost and, like Dr Wiesner, a fervent supporter of the college, speaks of the advantages of maintaining a "creative tension". But moves towards the policy direction — natural as they may be for an instititution with such close links to Washington policy-makers as MIT — have also exacerbated tensions with radical critics who, while intially keen that MIT should expand its efforts in the "science and society" area, now express concern about the way that this is being done. "It is certainly very important to study these issues, but establishing a research programme is no substitute for responsible social action on the part of scientists and technologists; the message of the college is that these controversies are matters for academic experts," says Dr Jonathan King, professor of biology and an active participant in recent DNA controversies.

Dr Blackmer expresses concern about the criticism that individuals who have been raising these issues on the campus have been excluded from the programme. But he defends this as the result of the need for a high level of academic credibility.

Here, too, however, there is a source of controversy. Dr Wiesner and Dr Rosenblith — both of whom have been asked to join the programme after their respective retirements next year — have publicly argued that universities can bring rationality to public debates about the risks of technological developments.

"University research, with its capacity to be comprehensive and with a credibility based on objectivity, can play an especially important role in studies aimed at clarifying for government the elements of conflicting social goals," says Dr Wiesner.

Critics, however, pointing to factors such as the sustained high profitability of chemical companies claiming to be overburdened by regulation, argue that the current 'debates' about risk are being partly managed as a public relations exercise by industry as part of a more general attack on social controls — and that MIT's academic credentials are being used to legitimate such tactics.

"There is a growing danger that, by recruiting university scientists to study these problems, private corporations are consciously trying to immunise themselves from public scrutiny by invoking both the scientists' stance of neutrality and objectivity, as well as the more general academic respectability of interdisciplinary programmes such as STS", says Dr David Noble, an associate professor of history.

Dr Stephan Chorover, professor of brain sciences and a long-term campus activist, puts it even more strongly: 'The bottom line is that the college is an exercise in what I call the engineering of consent: the product of a marriage between corporations with a large amount of money and low public credibility, and an institution that lacks the money but can provide the credibility."

Such criticism is not confined to plans for the college; another target is a new proposal, eagerly supported by some faculty members but criticised by others, that MIT should set up a "risk institute" largely at the behest of industry specifically concerned with technological risks and regulatory responses.

Given MIT's pre-eminence in what STS faculty member Dr Leo Marx has described as "the institutional embodiment of perhaps the major theme in the development of Western culture since, roughly, the seventeenth century", the outcome of the various debates will be watched with particular interest. ☐

news and views

Pathways to secretion

by Peter Newmark

OF the complex series of events that culminate in the secretion of products from cells, most can still only be classified as phenomenology in the view of G. Palade (Yale University), expressed at a recent meeting.* Although several attractive candidates are currently vying for recognition as molecular mechanisms of secretion, so far that accolade has only been conferred on the signal hypothesis.

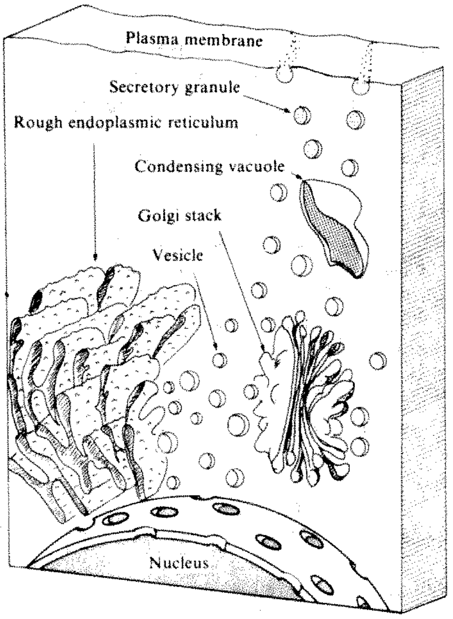
The signal hypothesis implies that proteins destined for secretion are synthesised with a sequence that guides them (and the ribosomes on which they are being synthesised) to the membrane of the endoplasmic reticulum (ER). As synthesis proceeds the nascent protein passes through the membrane into the lumen of the ER and from there, eventually, to and/or through the external membranes of the cell. Much evidence favours this concept, especially in the form in which the signal is a short, predominantly hydrophobic, amino-terminal sequence which is cleaved off as the protein traverses the ER. V. Lingappa (Rockefeller University) made the point that the concept could survive examples of non-terminal signals thereby rationalising the evidence he presented for the existence of an internal and non-cleaved signal sequence in ovalbumin (see *Nature* **281**, 117; 1979).

One prediction of the signal hypothesis is that the addition of a signal sequence to a non-secreted protein will lead it to be secreted. Another is that without the proper signal sequence a secretory protein will not be secreted. J. Beckwith (Harvard Medical School) described experiments with *Escherichia coli* mutants that have borne out both predictions. To test the first, genetic recombination was used to replace the amino-terminus of β-galactosidase (a cytoplasmic protein) with the amino-terminal sequences of either the maltose-binding protein (found in the periplasmic space between the inner and outer membranes of Gram negative bacteria) or the phage lambda receptor (an outer membrane protein). Either grafted signal sequence had the predicted effect of redirecting β-galactosidase from the cytoplasm to within, if not through, the bacterial membranes.

The *E. coli* with mutant β-galactosidase stuck in their inner cell membrane became phenotypically abnormal when induced with maltose. This led to confirmation of the second prediction of the signal hypothesis. For it was possible to isolate pseudorevertant bacteria which did not assume an abnormal phenotype when induced with maltose. These pseudorevertants did not have membrane-bound β-galactosidase. Genetic mapping showed this to be due to a mutation in the signal sequence region. Genetic recombination enabled the mutated signal sequences to be fused back to their normal proteins, maltose-binding protein or the lambda receptor; whereupon they were no longer secreted. Finally the exact mutations of the signal sequences that prevented their functioning were determined at the DNA level and sometimes at the amino acid level too. In most cases the change was of a hydrophobic to a charged amino acid.

Once across the rough endoplasmic reticulum and into the cisternal space, proteins are *en route* to secretion. But not all of them become secreted. In particular, certain enzymes are destined instead for the lysosomes. W. Sly (Washington University School of Medicine, St Louis) suggested that lysosomal enzymes are diverted from secretion by means of another signal, a mannose-6-phosphate group on a complex oligosaccharide group that is added to the enzymes in the cisternal space. The mannose-6-phosphate then acts as a recognition marker for receptors on the cisternal side of the endoplasmic reticulum membranes, the ligand-receptor complexes being internalised into vesicles which become lysosomes. These events, Sly believes, take place before the proteins reach the Golgi complex. If the mannose-6-phosphate recognition marker is missing from lysosomal enzymes they are secreted instead; this being the molecular basis of I-cell disease.

Although an exposed mannose-6-phosphate group may be unique to lysosomal enzymes, the presence of an oligosaccharide group is a common feature of secreted proteins. The groups are synthesised on a lipid (dalachol) carrier, added to the proteins during or shortly after translation, and processed before or in the Golgi complex (M. Snider, Massachusetts Institute of Technology). Although the role of these oligosaccharides is not clear it is possibly in the routing of the proteins to their final destination. A genetic approach to this problem may be found through the availability of temperature-sensitive mutants of vesicular stomatitis virus, the spike glycoproteins of which do not reach the surface of infected cells at the non-permissive temperature. Some of the glycoproteins do not acquire normal terminal sugars on their oligosaccharide side chains, although at least one does (H. Lodish, Massachusetts Institute of Technology).



Secretory pathways in the cell (adapted from *Functional Histology* (Wheater, Burkitt & Daniels) page 12, Churchill Livingstone).

Molecular mechanisms which are tentatively related to secretion were outlined by H. Pollard (NIH) and T. Stossel (Massachusetts General Hospital). Pollard described a protein, synexin, which can aggregate isolated chromaffin granules

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*Basic Mechanisms of Cellular Secretion, sponsored by the NIH and held in Annapolis, 17-21 September, 1979. A book based on the conference will be published by Academic Press in 1980.

Peter Newmark is Deputy Editor of Nature.

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in the presence of calcium. By analogy he believes that an influx of calcium into the cell would induce synexin-mediated fusion of chromaffin (or other) granules to the plasma membrane. For discharge of the contents of the granules another mechanism is needed. That, according to Pollard, involves the opening of an anion transport channel at the point of contact between the vesicle and plasma membrane leading to a chloride and energy-dependent chemiosmotic lysis of the granule. Stossel has a scheme whereby calcium activates the macrophage protein gelsolin (*Nature* **281**, 583; 1979) to reduce the length of the actin polymers of the cytoskeletal lattice. As a result he envisages that an influx of calcium could induce a local contraction of the lattice allowing access of the secretory granules to the plasma membrane.

Both of these speculative mechanisms involve triggering by an increase in the level of calcium ions, one of the best established features of stimulus-induced secretion. O.H. Petersen (Dundee University) described evidence from the pancreatic acinar cell that suggests the immediate release of calcium from an internal source in response to secretagogue stimulus. That calcium mediates the opening of ion channels for sodium and chloride, the influx of which accounts for the observed membrane depolarisation which is followed by secretion. Continued secretion requires the influx of calcium from an external source. That calcium may be brought into the cell by an ionophore. Candidate ionophores are phosphatidic acid (J. Putney, Wayne State University School of Medicine) and, at least in polymorphonuclear leukocytes, prostaglandin B_x (G. Weissmann, New York University School of Medicine).

The question of the route(s) of intracellular transport of secretory products from the rough endoplasmic reticulum to the plasma membrane remains vexed. The favoured scheme is one whereby vesicles are pinched off from the former and find their way to the distended ends of the Golgi stack. With these they fuse. What happens next is unclear but may involve passage of the secretory products to other sections of the Golgi complex, the GERL (several participants felt that this was no longer a useful concept) and the condensing vacuoles. Finally, from one or more of these morphological semi-entities, vesicles are again pinched off. These become the secretory granules as they head for the plasma membrane. What, if anything, guides or drives them there? One possibility for which the evidence is ambiguous, is that microtubules are involved. If so, it is conceivable that secretory granules are attached to the tubulin subunits of microtubules which, according to L. Wilson (University of California, Santa Barbara), are continually being added at one end, traversing the length and disassembling at the other end of microtubules of constant length. His latest evidence is that this 'treadmilling' is hastened by ATP, perhaps through the phosphorylation of one of the microtubule-associated proteins. The rate of treadmilling is thereby of the right order of magnitude were it to account for the conveyancing of granules to the surface of the cell.

Finally the secretory granule reaches the plasma membrane. Fission in the pancreatic B cell is preceded, according to L. Orci (University of Geneva) by the clearance of intramembranous particles and glycoproteins from the contiguous granule and plasma membranes. This is an observation common to many membrane systems but was disputed by T. Reese (NIH) for *Limulus* amibocytes observed with a time resolution of a few milliseconds. Orci also has very tentative evidence that the intramembranous particle-free membrane areas are rich in cholesterol, on the fiercely disputed assumption that it is aggregates of cholesterol which form the protuberances that are induced by addition of the antibiotic, filipin. These morphological correlates of exocytosis seem to be mirrored by an absence of filipin-induced protuberances and a concentration of intramembranous particles at sites of endocytosis on fibroblast membranes □

Jets in radio galaxies

from R.D. Davies

As higher angular resolutions become available to radio astronomers more evidence is coming to light on the important role of jets in the processes within radio galaxies and quasars. One of the first such objects to be discovered was the radio jet in the nearby radio galaxy Virgo A, also known as M87, the dominant galaxy of the Virgo cluster. This jet, seen at both optical and radio wavelengths, is a straight filament aligned precisely with the nucleus of M87; it lies to one side and extends 1.5 kiloparsecs (4,500 light years) from the nucleus. A similar geometrical arrangement occurs in the quasar 3C273, but on a larger scale; a straight jet projects 40 kiloparsecs from one side of the compact optical and radio quasar. The discovery of such jets lying relatively close to the nucleus of radio galaxies and quasars gave an impetus to the understanding of the huge outer radio emitting lobes. These are generally of similar intensity and lie a similar distance either side of a central object; the overall dimensions may be anywhere in the range of 100 to 3,000 kiloparsecs.

The arrangement of radio sources (it does not particularly matter whether they are radio galaxies or quasars) can be simply summarised. There are three main parts — a central power-house in which energy is released, an interconnecting region where energy is transmitted outwards and a large outer luminous region. Since these outer regions lose energy by radiation in a shorter time than it took them to expand out to their present position, the energy supply must be continually replenished. Therein lies the problem. The situation is analogous to the problem of supplying energy to a city from a distant coal-field. We might think of two ways of doing this. First, the energy could be converted directly into electricity then sent over transmission lines, or alternatively the coal could be transported by train and converted locally to the form of energy required. The same two alternatives have been proposed for radio sources. Rees has proposed that energy may be transmitted as intense low-frequency electromagnetic radiation which then penetrates to the outer lobes and causes them to radiate. Other theories have been conceived in terms of the mechanical transport of energy in which either cold gas or relativistic gas and magnetic fields are ejected at high speed from the nucleus and interact with the material in the outer lobes. The latter processes involving the mechanical transport of energy are more likely to produce observable radio jets.

New radio instruments with even higher angular resolution and sensitivity are coming into operation and are throwing light on the radio source problem. The pioneering instrument in this field is the Cambridge 5 km aperture-synthesis array; it has been for many years a major instrument in the study of structure of radio sources. It is now equipped with receivers at short wavelength to improve further its angular resolution. The Westerbork array in Holland is being increased in resolution by an extension of baseline to 3.2 km. The major development in this field in recent years is the Very Large Array (VLA) which is nearing completion in Socorro, New Mexico. The VLA will consist of 27 radio telescopes each 25 metres in diameter located on rail tracks in the form of a Y with each arm 18 km long. Higher resolution again will be achieved with the Multi-Telescope Radio-Linked Interferometer (MTRLI) at Jodrell Bank which will have telescopes distributed over baselines up to 133 km. The highest resolutions of all (milliarcseconds) are available with tape-recording interferometers on intercontinental baselines; these are particularly useful in probing the nuclear regions of radio sources.

The new observations of radio sources

R.D. Davies is Professor of Radio Astronomy at the Nuffield Radio Astronomy Laboratories, University of Manchester.

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with the partially completed VLA were a highlight of the recent General Assembly of the International Astronomical Union. 3C449 described by Perley, Willis and Scott (see *Nature* **281**, 437; 1979) is typical of the new data coming from the VLA. This is the radio source associated with a giant elliptical galaxy having a redshift of 5,400 km s^{-1}. The radio galaxy is typical of its class and has large outer radio lobes extending to 160 kiloparsecs on each side of the galaxy. What is particularly evident from the new results is the dominance of an inner jet structure lying symmetrically on either side of an unresolved nuclear source. These jets show a constant opening angle of about 7° which is most readily interpreted as arising from fast radial outward flow of emitting plasma in which there is also a transverse flow representing expansion at the speed of sound on the medium. A consistent picture emerges with a radial flow of about 1000 km s^{-1} which leads to a lifetime of 10^7 years for the bright inner jets.

In addition to the bright jets and the faint but extensive outer lobes there is an intermediate region of enhanced brightness. In all, there must have been three major outburst phases in 3C449, in each of which material was ejected outwards in opposite directions on more or less the same line. As the emitting plasma moves outwards it expands and becomes of lower surface brightness. Indeed the authors interpret the gap between the nucleus and the inner edge of the jet as indicating that there may have been an interval since the last outburst of a few million years. These observations clearly favour an explanation in terms of a mechanical flow of energy in the form of a plasma of relativistic particles and magnetic fields. The details of the geometry, the polarisation and the spectral index of the emission along the jets will be important in the full understanding of the physical mechanisms in radio galaxies. ☐

Bent DNA

from Michael Spencer

FOR many years after the initial excitement wore off, the devotees of DNA stereochemistry soldiered on with their studies amid some disbelief that the detailed results would be of much interest to those working on biochemical problems. It was an esoteric speciality, occupied with the niceties of sugar ring pucker, base tilt and base twist. All this began to change as biochemists accumulated evidence for non-coding regions of DNA containing special sequences, in particular the promoter region associated with the binding of RNA polymerase. It appeared in this case that the binding caused a localised unwinding of the DNA duplex, and interest grew in discovering exactly what changes in structure were involved. In this way it was hoped to obtain a better understanding of how the enzyme recognised the site and initiated synthesis.

In two other fields DNA deformation became of interest, and models were proposed for a 'kink' involving the unstacking of one base pair relative to an adjacent pair. It seemed a plausible way of explaining the compaction of DNA in the nucleosome (Crick & Klug *Nature* **255**, 530; 1975), though other interpretations were later suggested (see *News and Views*, 1 September 1977). In the field of DNA-drug interactions, it was proposed that intercalation of a drug molecule could occur at sites where a spontaneous kink, induced by a thermal energy fluctuation, allowed the drug to slip in between the base pairs (Sobell *et al. Cold Spring Harbor Symp. quant. Biol.* **42**, 87; 1977). It was further suggested that such a kink might propagate down the polymer in a wave like disturbance.

In all this, it was generally assumed that DNA remained in the well-known *B* conformation that purified material shows in an aqueous environment. However, a few workers always hoped that they would some day find a biological role for the rather different *A* conformation that is often found under conditions of low water content and low ionic strength. This has 11 instead of 10 base pairs per turn, and the bases are tilted and laterally displaced in relation to the helix axis. It has been known since 1962 that RNA had a structure of the *A* type, and the *A* structure received a further boost when it was discovered that DNA-RNA hybrids preferred it. The idea germinated that even if DNA *in vivo* did not adopt an *A* structure along its whole length, there might be local transitions induced by changes of environment or interaction with other, more hydrophobic molecules (Arnott *et al. Nature* **220**, 561; 1968, Florentiev & Ivanov *Nature* **228**, 519; 1970). More recently, Minyat *et al.* (*J. molec. Biol.* **128**, 397; 1978) have obtained evidence from circular dichroism that spermine and spermidine do stabilise the *A* conformation in solution, under conditions of decreased water activity. They deduced that the cooperative unit could be as short as 10 base pairs.

A collaboration between groups at Wisconsin and Purdue (Selsing *et al. J. biol. Chem.* **254**, 5417; 1979) has now led to a convincingly detailed model for a DNA duplex containing an *A* structure at one end and a *B* structure at the other, with a sharp bend in the middle where the conformation changes. Their work follows the synthesis of a model copolymer containing dG along the whole of one strand; the other strand contains a block of rC, giving the *A* structure characteristic of hybrids, linked by T4 DNA ligase to a section of dC, which in solution leads to a *B* structure. Solution studies by Selsing and Wells (*J. biol. Chem.* **254**, 5410; 1979) have confirmed the earlier conclusion from NMR work that the transition region is confined to only one or two base pairs.

The new model-building exercise was carried out for a purely DNA duplex with only a single nucleotide pair in the transition region. In the most satisfactory of the models tested, the transition base pair has one nucleotide with the sugar pucker characteristic of *A*-DNA, and one with the different pucker found in *B*-DNA. The change of conformation leads to a 26° change in the direction of the helix axis, while preserving full base stacking and hydrogen bonding. It may thus be energetically preferable to an unstacked kink. Furthermore, the base stacks predicted at the junction for different DNA sequences have noticeable similarities to those actually found in dinucleotide and polynucleotide structures.

The authors discuss the biological implications with some enthusiasm. Their model gives respectability to the belief that segments of *A*-DNA may be induced to form in an otherwise *B*-DNA helix, and this might well explain the observed unwinding of DNA on binding RNA polymerase. However, they do not go into the question of how the RNA might then be copied; does the helix open up, as originally suggested by Florentiev and Ivanov, or is a triplet structure formed as suggested by Riley (*Nature* **228**, 522; 1970)? Another relevant matter which they do mention is the discovery of alternating A, T-rich and G, C-rich blocks in many regulatory regions. In DNA fibres, the former type of sequence leads to a preference for a *B* conformation, while the latter extends the stability range of the *A*. If these preferences held under cellular conditions (which remains to be proved), the DNA could adopt a tertiary structure determined by the length of the segments. Alternating single turns of each kind of helix, both containing about 10 base pairs, would result in the helix axis following a zigzag path. On the other hand, half a turn of each helix would result in a small loop of DNA, and the authors illustrate a computer-generated model for such a structure.

If such features were stable *in vivo* they might form recognition sites for protein ligands; alternatively, they might be induced to form by the more hydrophobic environment resulting from the presence of protein. It is an intriguing thought that an enzyme working its way down the DNA duplex might trigger off a local change in conformation that stopped it in its tracks. The hunt must now be on for direct evidence of such local changes in structure. The authors point out that a loop containing *A*-DNA segments will contain a distinctly shorter average length per nucleotide than the superficially similar structure obtained by smoothly bending *B*-DNA. This could, they say, explain a

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Michael Spencer is a Research Fellow in the Department of Biophysics at King's College, London.

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recent observation by Griffiths (*Science* **201**, 525; 1978) that electron micrographs of ϕX174 DNA suggest a nucleotide repeat of only 2.9 Å, instead of the 3.4 Å expected for *B*-DNA. The alternative explanation preferred by some workers is the existence in solution of a *B*-like structure with slightly more than 10 residues per turn (Wang *Proc. natn. Acad. Sci. U.S.A.* **76**, 200; 1979).

The authors finish with a flourish of speculation about the possibility of travelling disturbances. The idea that DNA can 'breathe' and develop a travelling region of denaturation is an old one, and the idea of travelling kinks has already been mentioned; it now seems possible that travelling discontinuities between regions of different DNA conformation might be important in biological recognition. The sequence-specific conformational preferences that have been shown for DNA would, they say, act as 'sources, sinks and barriers' for transient conformational discontinuities in the helix. The idea of DNA as an inert repository of genetic information seems really to have breathed its last. □

Locust phase colouration

from Richard J. Moore

CHANGE in colouration is the most noticeable feature of the 'phase' transformation which locusts undergo during their large population fluctuations, yet its adaptive significance has proved one of the most difficult to explain. Individuals in low population densities during plague recessions (*solitaria* phase) are a uniform green, presumably a disguise from the attentions of visually-guided predators. During plague outbreaks, individuals develop a striking black and yellow or orange colouration (*gregaria* phase).

This suggests an aposematic or warning function to predators; yet locusts are voraciously pursued by flocks of birds, and nomads regard them as something of a delicacy. Batesian mimicry of a poisonous or distasteful model is unlikely to be operating since no possible models with a similar range are known, and in any case the vast numbers of locusts in swarms would greatly outnumber any model.

Early research on the problem focused on the possible role of the *gregaria* colour pattern in maintaining migratory groups. In 1928, Boris Uvarov suggested that the increased activity of hoppers (nymphs) in bands resulted from an optomotor or visual compensation response to movement of neighbours. Peggy Ellis (*J. exp. Biol.* **30**, 214; 1953) found, however, that a background of moving vertical stripes was not as effective in maintaining the characteristic 'marching' of hoppers as visual stimuli from moving locusts. This and a more recent experiment by Sylvia Gillett suggests that locust shape is the more important stimulus for grouping. Gillett (*Anim. Behav.* **21**, 153; 1973) mixed normally-pigmented *gregaria* desert locusts with albinos, which aggregate less than coloured individuals. Mixing them neither reduced the grouping of normal locusts, nor increased the grouping of albinos: thus the reduced grouping of albinos alone must be due to some other factor besides lack of colouring.

It should be noted that Gillett's experiment examined static aggregation; and also that the vertical stripes in Ellis's experiment were not specifically designed to imitate *gregaria* locust patterning. So it remains a possibility that the patterning has evolved to maximise the optomotor response, and thus cohesion of moving groups.

Recently, however, workers have begun to examine the role of predation as the evolutionary force promoting phase polymorphism; indeed, aggregation itself may give a defensive advantage against predators. Matthews proposes (*Amer. Nat.* **111**, 213; 1977) that individual locusts, and also Australian stick insects (which undergo remarkably similar density-dependent phase transformations), contain small amounts of toxins, so that the consumption of large numbers of them by a predator would eventually exceed its tolerance limit. Remembrance of the strikingly-coloured prey which had produced the intoxication would result in a specific avoidance image (a negative 'search image') preventing further consumption of locusts. It is doubtful, though, how much protection would be conferred in this way, since large birds such as storks are known to be capable of consuming in the order of 1000 adult locusts per day, day after day.

Gillett has begun to look at the factors affecting locusts' vulnerability to predation. Against a green irregular background, surprisingly, *gregaria* nymphs are just as effectively camouflaged as *solitaria* (Gillett & Gonta *Anim. Behav*, **26**, 282; 1978). But in a strategy of crypsis, density is also of major importance, as her experiments showed. In the wild, *gregaria* nymphs often occur massed in numbers so great as to preempt any possibility of escaping detection. These very numbers, and their increased activity, might make it difficult for a predator to pick off individual locusts. Could the *gregaria* pattern contribute to this?

In her latest work (Gillett, Hogarth & Noble, *Anim. Behav.* **27**, 592; 1979), Gillett and her coworkers offered *gregaria* desert locusts at a range of densities to a lizard and human predators in an otherwise empty arena. The catching efficiency of the human predators did not vary much, but the effect of increasing prey density on the lizard's catching efficiency was dramatic: above a certain prey density, the lizard became totally incapable of catching any locusts. Gillett *et al.* attribute these results to a 'visual confusion effect' produced by the activity of the locusts — an effect which, they suggest, would be enhanced by the bold patterning. This remains to be demonstrated, but there are strong indications of the importance of prey-striping in other predator — prey interaction (see, for instance, the discussion in Milinski, *Z. Tierpsychol.*, **45**, 373; 1977).

Although this is an attractive hypothesis, it may still not be the whole story. There is no reason why the *gregaria* colour pattern should not have multiple functions, but it is remarkable that neither of the most likely functions in locusts — group cohesion and predator confusion — could be of any use to Australian stick insects: they do not aggregate, and even at high densities, are very inactive. □

Richard Moore is a member of the Cambridge C.S. Research Group, a contract research organisation specialising in tropical marine conservation.

New human myeloid leukaemia cell line undergoes red shift

from P.R. Harrison

BIOLOGISTS interested in elucidating how cells acquire their specialised functions (the process of cell differentiation) are always on the look out for ways in which these normal developmental processes can be mimicked by *in vitro* systems as these often allow the design of more sophisticated experimental approaches than are normally possible *in vivo*. This applies with even greater force of course to experiments with human cells. For a long time, cells from leukaemic patients or animals have provided useful models for how cells grow and differentiate and the relationship between the occurrence of malignancy and consequent abnormal cell growth and differentiation.

Recently a new human cell line has made something of an impact in the literature on account of its very interesting properties. The K562 cell line was established several years ago by Lozzio and Lozzio working at the University of Tennessee from the pleural effusion of a patient with chronic myeloid leukaemia (CML) in terminal blast crisis (*Blood* **45**, 321; 1975). Initially it was assumed, largely on morphological criteria, that the K562 cells were derived from the outgrowth of a malignant clone of myeloid leukaemic cells arrested at some very early stage of granulocyte

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P. R. Harrison is at the Beatson Institute for Cancer Research, Glasgow.

differentiation. By analogy with the now classic experiments in which mouse erythropoietic cell precursors infected with Friend virus can be induced to differentiate further by treatment with DMSO and other chemicals (Friend *et al. Proc. natn. Acad. Sci. U.S.A.* **68,** 378; 1971). Lozzio's group tested the effect of DMSO on the human K562 cell line but could find no evidence of enhanced differentiation (Bamberger *et al. Leukemia Res.* **2,** 305; 1978).

But this year the situation has changed dramatically with a series of recent reports. First, Anderson and his colleagues at the University of Helsinki found to their surprise that in fact the surface properties of the K562 cell line resembled red blood cells rather than granulocytes or their benign or leukaemic precursors and in particular K562 cells were found to synthesise a highly specific red blood cell membrane protein, glycophorin (*Int. J. Cancer* **23,** 143; 1979; *Nature,* **279,** 498; 1979). Further support for the idea that K562 cells are in fact erythroid precursor cells comes from the subsequent finding by Anderson and his colleagues that sodium butyrate can induce erythroid maturation in K562 cells as judged by the formation of erythrocyte-like particles and haemoglobin, assayed either by benzidine staining or immunologically (*Nature* **278,** 364; 1979). This conclusion has recently been taken one important and very interesting step forward by Rutherford and his colleagues in Oxford who have reported that haemin induces human embryonic haemoglobin in K562 cells, as judged by starch gel electrophoresis and comparison of fingerprints of tryptic peptides with those from authentic human embryonic haemoglobins (*Nature* **280,** 164; 1979).

Whether other inducers (for example, butyrate in the experiments of Anderson *et al.*) induce embryonic or adult haemoglobin in K562 cells is not yet clear. There is some precedent for this in the Friend cell where haemin induces preferentially a minor ß-globin chain, rather than the usual major ß-globin chain (Rovera *et al. FEBS Lett.* **81,** 366; 1977; Nudel *et al. Proc. natn. Acad. Sci. U.S.A.* **74,** 1100; 1977). Certainly, if different inducers were to induce different globin chains, then the K562 cell would provide an extremely interesting system for studying the embryonic to adult globin gene switch *in vitro.*

Nevertheless, the originators of the K562 cell line are evidently still not at all happy with this accumulated evidence for its erythroid nature since they cannot reproduce the induction of haemoglobin in their own original stocks of K562 cells by sodium butyrate which in their hands is merely cytotoxic (Lozzio *et al. Nature,* **281,** 709; 1979). Perhaps this is not too surprising since Rutherford *et al.* (*op. cit.*) found haemin to be the only effective inducer of K562 cells, butyric acid and other Friend cell inducers tested being ineffective at the concentrations normally used. But those familiar with the Friend cell field will not be too perturbed at these differences between the laboratories concerning which inducers are most effective, since precise inducer concentrations and other variables in the culture conditions are often important. For example the batches of serum used, particularly in experiments where butyric acid is used as inducer, can affect the outcome. In any event, Lozzio *et al.*'s suggestion that the cells used by Anderson *et al.* (and presumably also Rutherford *et al.*) may have been contaminated with other cells with erythroid properties (a claim hotly and justly denied by Anderson *et al.* on the basis of karyotype evidence — *Nature,* **281,** 710; 1979) seems to me to be an inadequate explanation. Whether Anderson's karyotypically-authentic K562 line might have acquired its erythroid properties during clonal evolution in culture rather than *in vivo* is more difficult to resolve.

What then is the significance of the K562 cell line for the future? Is it a matter of *déjà vu* and essentially a repeat of the Friend cell story in a human context? Not simply. The isolation of human leukaemic cell lines other than those derived from lymphoid precursors is relatively rare and therefore interesting especially when an adult cell can be induced to produce embryonic-type proteins. Since embryonic haemoglobins have not been found in association with CML the controversial origin of the cell is clearly of some considerable interest *per se.* Further, the existence of cell lines producing embryonic and adult globins (the K562 and Friend cells respectively) may allow the differential regulation of the embryonic and adult globins to be studied more thoroughly, for example, possibly by exploiting the effects of different chemical inducers or by cell fusion studies. And undoubtedly the K562 cell line will be exploited simply because it is a human erythroid cell line, for example it can be used for studies of the properties of human red blood cell precursors and as a source of embryonic globins and other red blood cell proteins. ☐

Pituitary growth hormone

from Mike Wallis

THE pituitary growth hormones (GHs) together with the prolactins and placental lactogens comprise a family of protein hormones which regulate somatic growth, lactation and various other biological functions. An international symposium on GH and related hormones is held at four-yearly intervals in Milan; the most recent one* was broadened to include 'Other Biologically Active Peptides' though fortunately this was not taken too literally, and GH and its relatives remained the main focus of attention (and will be the only topic considered here).

As in many other areas of biology, recombinant DNA technology is making a major impact on polypeptide hormone biochemistry. H.M. Goodman (University of California, San Francisco) described the cloning and characterisation of the gene for human GH. The gene includes at least two introns, and some evidence suggests that several slightly different (non-allelic) copies of the GH gene may exist in the human genome. The nucleotide sequence of the (cloned) cDNA has been determined, including the entire coding region for the preGH. Expression of immunologically active human preGH (as a fusion protein including part of the bacterial trpD gene product) has been achieved in *E.coli* and the production of substantial quantities of human GH by genetic engineering in bacteria must now be imminent. The timing is appropriate since the hormone may well have new clinical uses (in addition to treatment of hypopituitary dwarfism); V. Zumtobel *et al.* (University of Munich) described applications of the hormone in the treatment of stress ulcers.

Another technique with great potential was described by J. Ivanyi (Wellcome Research Institute, Beckenham, UK) who reported the preparation and application of monoclonal antibodies to human GH. At least two antigenic sites can be recognised on human GH, only one of which is shared with human placental lactogen. This powerful technique should provide both specific probes for investigating structure-function relationships and reagents with increased specificity for radioimmunoassay.

Lack of detailed information about the three-dimensional structure of any member of the GH-prolactin family is a major drawback in understanding their biochemistry. The main problem is lack of suitable crystals for crystallography, and the reasons for this were discussed by K. Moffat (Cornell University). Crystals of human placental lactogen have been obtained but were insufficiently ordered for X-ray analysis. Heterogeneity of the hormone preparations that have been used for crystallisation attempts is a major problem, and Moffat urged those present to try and purify further, and to crystallise,

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*An international symposium on 'Growth Hormone and other Biologically Active Peptides' was held in Milan on 17-19 September. The meeting was organised by A. Pecile, E.E. Muller and M.L. Pecile and the Chairman was C.H. Li. Abstracts have been published (*Ricerca Scientifica ed Educazione Permanente* Supplement 11) and invited papers will be published in full by *Excerpta Medica.*

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their favourite hormones.

In the absence of information about three-dimensional structure, knowledge about structure-function relationships must come from other sources. Species specificity is marked among the GHs (thus, non-primate growth hormones are not active in man) and comparisons of GHs from different species may eventually provide clues to structure-function relationships. Purification and characterisation of GHs from various lower vertebrates was described by H. Papkoff *et al.* (University of California, San Francisco). Immunological and physicochemical differences between the hormones mainly reflect the extent of phylogenetic divergence, but biological activities are rather variable and do not follow phylogenetic relationships so closely. Sequence information for these proteins is eagerly awaited, but for the present our knowledge of the molecular evolution of this protein family remains based largely on the sequences of mammalian hormones. Studies on chemically modified forms of GH, and on synthetic fragments, may also help relate structure to function, and were described by A.C. Paladini *et al.* (University of Buenos Aires) who are attempting to define precisely which residues are required for activity. At least one antigenic site has been tentatively identified as associated with a region of 30–50 amino acid residues long, near the centre of the primary structure.

The mechanism of action of GH remains unclear. In particular it remains uncertain (and a matter of considerable controversy) as to which of the actions of the hormone are direct and which are mediated by somatomedins. Like other polypeptide hormones, GH is thought to bind initially to a membrane receptor, and the nature and characterisation of receptors was discussed in several papers. Direct actions of the hormone on erythroid colony formation *in vitro* were described by D.W. Golde (University of California, Los Angeles) and direct actions on amino acid uptake and incorporation by diaphragm *in vitro* were discussed by K. Albertsson-Wikland, A. Lindahl and their colleagues (University of Göteborg).

However, most of the discussion about the actions of GH centred on the somatomedins and their role in mediating these actions. Various forms of somatomedin have been described, at least some of which are very similar to the insulin-like growth factors IGF 1 and IGF 2, which have now been quite well characterised and which were discussed by J. Zapf and E.R. Froesch (University Hospital, Zurich). J.J. Van Wyk *et al.* (University of North Carolina) described studies on the characterisation of human somatomedin C, including determination of a partial amino acid sequence; the hormone is similar to IGF 1. Somatomedin C stimulates growth of various types of cell *in vitro* and was also shown to have *in vivo* actions on the frog lens epithelium. Studies on somatomedin A were described by A. Skottner *et al.* (A.B. Kabi, Stockholm) though amino acid sequence data are still not available for this hormone, and its relationship to the IGFs is uncertain. Somatomedin B, is now thought to be without somatomedin-like properties, and therefore inappropriately named. Although somatomedins A and C are thought to mediate at least some of the actions of GH, and although they have many biological effects *in vitro*, attempts to show GH-like actions of somatomedins *in vivo* have previously met with little success. However, J.V.L. van den Brande, S.C. van Buul-Offers and their colleagues (Wilhelmina Children's Hospital, Utrecht) reported that clear-cut growth hormone-like effects (including increase in body weight and length) have now been obtained when human somatomedins were injected into hypopituitary dwarf mice. Somatomedins are thought to be produced in the liver in response to stimulation by GH and other factors. Studies on the biosynthesis of somatomedin in cultured Buffalo Rat Liver cells were described by S.D. Schalch *et al.* (University of Colorado) who presented evidence for a precursor of the hormone of molecular weight about 32,000. Other hepatocyte systems which produce somatomedins (and also their binding proteins) were also described (G. Haselbacher *et al.*, University of Zurich; M. Binoux *et al.*, Trousseau Hospital, Paris); stimulation of somatomedin synthesis by GH was claimed. ☐

Mike Wallis is a Lecturer in Biochemistry at the University of Sussex.

The physics of biology

from H. Eisenberg

THE business-like mood at EMBL's Hamburg laboratory at the DESY synchrotron installation was a welcome change from the *fin-de-siècle* recreational workshop atmosphere one sometimes encounters at fashionable watering places. At the recent EMBO-EMBL workshop* held there, hope, progress, expectation, and fulfilment in the biological applications of neutron and X-ray scattering, in particular with synchrotron radiation, were discussed in depth.

The ILL neutron reactor in Grenoble has now been active for several years and an EMBL outstation, to provide the infrastructure for experiments in biology, was established some years ago. Synchrotron radiation provides information additional and complementary to the neutron scattering and, in particular, the high intensity and pulsed nature of the beam presents unique opportunities. The EMBL laboratory in Hamburg, directed by Heinrich Stuhrmann, has made great strides in the continuing development of suitable facilities to explore these opportunities. Also, construction of new facilities for chemistry and biology at the DORIS storage ring in Hamburg is now underway and, in large measure, success will depend on the concomitant development of appropriate two-dimensional fast detector systems.

Sessions at the workshop reflected the varied interests of the workers involved. Muscle and collagen are two systems long studied by both X rays and neutron fibre diffraction. In particular, hope was expressed that considerable reduction of data collection time into the millisecond range, in the case of synchrotron radiation, will enable performance of dynamic studies and characterisation of individual states in the contractile systems. In solution studies a major tool is the application of contrast variation, particularly favoured by the dramatic difference in neutron scattering between hydrogen and deuterium (with little change in structural or functional properties); complementary information is provided in the case of X-ray scattering by the use of the more classical contrast variation media such as sucrose or salts. These and other theoretical aspects were discussed, as well as the possibility of obtaining information-rich three-dimensional reconstructions from the analysis of spatial fluctuations from random systems. This should be useful for complex biological systems which have not been obtained in crystalline organised form, useful for X-ray diffraction. Problems related to anomalous scattering were also discussed.

Protein–nucleic acid interactions were discussed in sessions devoted to progress in the study of ribosomes, chromatin and viruses. In chromatin we are gaining more understanding on the higher order structures of solutions of finite chains of nucleosomes in a variety of experimental conditions. Additional major topics of discussion involved membranes and lipoprotein complexes, as well as proteins and protein assembly complexes, such as microtubule structure.

The close encounter between about eighty molecular biologists, theoreticians and instrumentalists led to a frank exchange of ideas, created new and extended existing paths of communication in a field of great topical interest.

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*The second EMBO-EMBL workshop on 'X-Ray and Neutron Scattering of Biological Structures' took place in Hamburg on 24-28 September, 1979.

H. Eisenberg is in the Polymer Department, Weizmann Institute of Science, Rehovot, Israel.

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articles

Distortions of the cosmic microwave background spectrum by dust

M. Rowan-Robinson*†, J. Negroponte† & Joseph Silk†

* Queen Mary College, London E1, UK
† University of California, Berkeley, California 94720

The cosmic microwave background spectrum supports the idea of a pre-galactic generation of stars. The distortions of the background spectrum from a black body can be due to emission by dust if a modest fraction of the mean cosmological density is present as silicate particles at epochs corresponding to redshift $z \gtrsim 100$. Whether the entire background can be explained as emission from dust is investigated.

IN the standard hot big bang cosmology, the Jeans mass at the epoch of decoupling of matter and radiation ($z \sim 1,000$) is about $10^6\,M_\odot$, so that any strong density perturbations present on this scale would collapse rapidly, in $\sim 10^6[(1+z)/1,000]^{3/2}$ yr. Dicke and Peebles[1] proposed that globular clusters might have formed in this way before galaxies did. White and Rees[2] suggested a more radical version of this idea, in which an early generation of stars, perhaps formed by these $10^6\,M_\odot$ objects, evolved rapidly to form dark remnants (presumably black holes or stars of very low mass) and now comprise the 'missing mass' postulated to bind clusters of galaxies and to make up haloes around massive spiral galaxies[3]. The concept of a pre-galactic generation of stars, often referred to as 'Population III', was taken further by Rees[4], who suggested that the cosmic microwave background radiation could be the light from such stars absorbed and re-thermalised by dust grains, molecules or ionised gas. Various earlier schemes[5-13] for a non-cosmological origin of the cosmic background radiation have encountered difficulties, because of the severe requirements of energetics and thermalisation. The hypothesis of Rees minimises these problems, and may lead to an explanation of the observed average number of photons per baryon in the Universe, an unexplained constant ($\sim 10^9$) in the standard big bang models.

The effect of dust in the early Universe

We investigate here the effect of dust in the early Universe on the cosmic microwave background, including the effect of a pre-galactic Population III generation of stars. We assume that at epochs such that $z \gg 100$, the cosmic background has a black-body spectrum of temperature $T_r = T_0(1+z)$. In Rees' model[4], $T_0 \ll 3$ K, but in more conventional models $T_0 \sim 2.7$–3.0 K. Suppose that at an early epoch a generation of stars is born that live for a time t_s, dying at epoch t_f, where $t_s \ll t_f$.

Let ε be the efficiency with which the matter in the Universe is converted into radiation by this Population III. If most of the matter in the Universe forms into Population III objects and undergoes conversion to helium and heavy elements, then ε can be as high as ~ 0.01. If most of the energy is generated by accretion of material onto black holes, then ε could be as large as 0.1–0.3. Conventional models for quasars evidently require such a high efficiency[14].

Assuming the Population III objects radiate as black bodies with temperature T_s, radius r_s, and have number density η_s at epoch t_f, then

$$\varepsilon\rho_m(t_f)c^2 = 4\pi r_s^2 \eta_s \sigma T_s^4 t_s \qquad (1)$$

where $\rho_m(t)$ is the matter-density at epoch t.

The energy-density in radiation is enhanced by this radiation input by a factor

$$\begin{aligned}\beta &= \frac{\varepsilon\rho_m(t_f)c^2}{aT_0^4(1+z_f)^4} = \frac{\varepsilon\rho_m(t_0)c^2}{aT_0^4(1+z_f)} \\ &= 420\varepsilon\Omega h^2[100/(1+z_f)](2.7/T_0)^4\end{aligned} \qquad (2)$$

where Ω is the cosmological density parameter, and h is the Hubble constant (units of 100 km s^{-1} kpc^{-1}).

Suppose that these stars give rise at their death to a heavy element abundance Z throughout the remaining gas in the form of dust. Presumably $Z < 10^{-3}$ for consistency with the lowest values found in extreme Population II (ref. 15). If the dust is uniformly distributed, the resulting optical depth at epoch t_1 to a source emitting at epoch t_2,

$$\tau_\nu(t_2, t_1) = \pi a^2 c \int_{t_2}^{t_1} n_g(s) Q_{\nu(1+z)}\, ds \qquad (3)$$

where $n_g(t)$ is the number-density of grains at epoch t, and $1+z = R(t_1)/R(s)$. Thus

$$\tau_\nu(t_2, t_1) = A \int_{t_2}^{t_1} Q_{\nu(1+z)}[R_0/R(s)]^3\, ds/t_H$$

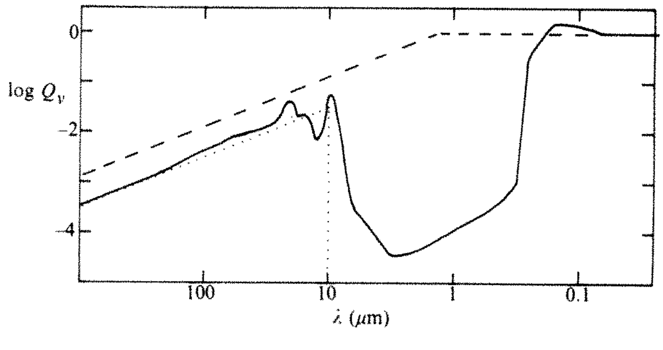

Fig. 1 Dust absorption efficiency used in this work. Solid line, amorphous silicates; dotted line, analytic approximation to these (equation (15)); dashed line, equation (14).

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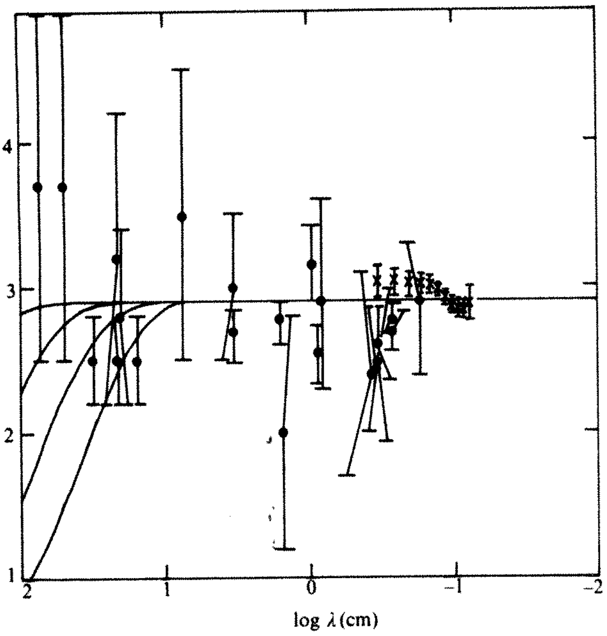

Fig. 2 Observed microwave background spectrum (●, from Danese and de Zotti[13]; ×, from Woody and Richards[20]), plotted as thermodynamic temperature, $T_{\rm th}$, against wavelength, compared with predictions for grains with Q_ν given by equation (14), $T_{\rm d}=2.9$, $T_0=0$, $1+z_{\rm f}=200$ and (from the top) $qZ=10^{-2}$, 4×10^{-3}, 2×10^{-3}, 10^{-3}.

where $A=\pi a^2 n_{\rm g}(t_0)ct_{\rm H}$, Q_ν is the absorption efficiency of the grains (as the radiation field in this problem is always isotropic, scattering can be entirely neglected), $R_0=R(t_0)$, and $t_{\rm H}$ is the Hubble time at the present epoch t_0.

In terms of Ω,

$$A=5.1\,\Omega h\,(Z/0.001)(0.1\,\mu{\rm m}/a)(2.5\,{\rm g\,cm^{-3}}/\bar\rho_{\rm g}) \tag{4}$$

where a is the grain radius, and $\bar\rho_{\rm g}$ is the mean density of the grain material.

If $T_{\rm d}(t)$ is the temperature of the dust grains at epoch t, the intensity of radiation observed at epoch $t>t_{\rm f}$ is the sum of the contributions from the cosmic background and Population III, attenuated by dust, and from the emission by dust:

$$\begin{aligned}I_\nu(t)=\{&(1+z_{\rm r}')^{-3}B_{\nu(1+z_{\rm r}')}[T_{\rm r}(t)]\\&+(1+z_{\rm f}')^{-3}\pi\eta_{\rm s}r_{\rm s}^2ct_{\rm s}B_{\nu(1+z_{\rm f}')}(T_{\rm s}\}\exp\left(-\tau_\nu(t_{\rm f},t)\right)\\&+\int_{t_{\rm f}}^{t}(1+z')^{-3}B_{\nu(1+z')}[T_{\rm d}(s)]\exp-\tau_\nu(s,t)\,{\rm d}\tau_\nu(s,t)\\&\qquad\qquad{\rm erg\,cm^{-2}\,s^{-1}\,H^{-1}}\end{aligned}$$

where $1+z_{\rm r}'=R(t)/R(t_{\rm r})$, $1+z_{\rm f}'=R(t)/R(t_{\rm f})$, $1+z'=R(t)/R(s)$. This can be written:

$$\begin{aligned}I_\nu(t)=\{&B[T_0(1+z)]+\pi\eta_{\rm s}r_{\rm s}^2ct_{\rm s}B_\nu[T_{\rm s}/(1+z_{\rm f}')]\}\\&\times\exp-\tau_\nu(t_{\rm f},t)\\&+\int_{t_{\rm f}}^{t}B_\nu[T_{\rm d}(s)/(1+z')]\exp-\tau_\nu(s,t)\,{\rm d}\tau_\nu(s,t)\end{aligned} \tag{5}$$

where $1+z=R_0/R(t)$.

The equation of radiative balance for the grains takes the form

$$\int_0^\infty Q_\nu B_\nu[T_{\rm d}(t)]\,{\rm d}\omega=\int_0^\infty Q_\nu I_\nu(t)\,{\rm d}\nu \tag{6}$$

In particular, at $t_{\rm f}$ equation (6) becomes

$$\begin{aligned}\int_0^\infty Q_\nu B_\nu[T_{\rm d}(t_{\rm f})]\,{\rm d}\nu=&\int_0^\infty Q_\nu B_\nu[T_0(1+z_{\rm f})]\,{\rm d}\nu\\&+\pi\eta_{\rm s}r_{\rm s}^2ct_{\rm s}\int_0^\infty Q_\nu B_\nu(T_{\rm s})\,{\rm d}\nu\end{aligned} \tag{7}$$

As $T_{\rm d}(t_{\rm f})\leqslant T_{\rm m}$, the melting temperature of the grains, equation (7) implies

$$\pi\eta_{\rm s}r_{\rm s}^2ct_{\rm s}\int_0^\infty Q_\nu B_\nu(T_{\rm s})\,{\rm d}\nu<\int_0^\infty Q_\nu B_\nu(T_{\rm m})\,{\rm d}\nu \tag{8}$$

Assuming the stars radiate in the UV (for example, 0 stars with $T_{\rm s}\sim40{,}000$ K) so Q_ν can be set equal to unity on the left hand side, equation (8) is equivalent to a limit on the product $\beta(1+z)^4$. If we set $Q_\nu=2x$, $x=2\pi a/\lambda$, we find $\beta(1+z_{\rm f})^4<10^{10.65}(T_{\rm m}/1{,}500)^5$. If $\beta=1$, as in Rees' model[4], $(1+z_{\rm f})<400$. Before this epoch grains could not survive. For silicate grains, the limit is more severe, $1+z_{\rm f}<10^{2.03}\,\beta^{-1/4}$ for $T_{\rm m}=1{,}500$ K.

High optical depth limit

Suppose that at the present epoch, the optical depth in dust is large at all wavelengths ⩽20 cm, say. Then the contribution of the first two terms on the right hand side of equation (5) to the integral on the right hand side of equation (6) is negligible, and the solution of equation (6) is

$$T_{\rm d}(t)=T_{\rm d}(t_0)(1+z),\qquad t\gg t_{\rm f} \tag{9}$$

The observed intensity at the present epoch in the microwave range would be

$$\begin{aligned}I_\nu(t_0)=B_\nu(T_0)\exp-\tau_\nu(t_{\rm f},t_0)+B_\nu[T_{\rm d}(t_0)]\\\times[1-\exp-\tau_\nu(t_{\rm f},t_0)]\end{aligned} \tag{10}$$

To determine $T_{\rm d}(t_0)$, we note that the conservation of co-moving energy density implies that

$$T_{\rm d}(t_0)^4\simeq T_0^4(1+\beta) \tag{11}$$

In particular if there were no radiation from stars ($\beta=0$), then $T_{\rm d}(t_0)=T_0$, and $I_\nu=B_\nu(T_0)$, so that there is no distortion from a black-body spectrum. Equation (9) may not be a bad assumption where the optical depth in the UV is high, but that in the microwave region is ⩽1. This is because although $T_{\rm d}(t_{\rm f})\gg T_0(1+z_{\rm f})$ in general, the temperature subsequently drops rapidly with time as $\tau_{\rm UV}(t_{\rm f},t)$ increases, until it is of the form of equation (9). However, in this case equation (11) should be replaced by

$$T_{\rm d}(t_0)^4[1-\exp-\tau_{\nu(T_0)}]\simeq T_0^4\,[1-\exp-\tau_{\nu(T_0)}+\beta) \tag{12}$$

where $\tau_{\nu(T)}$ is the optical depth back to $t_{\rm f}$ at the frequency corresponding to the peak of a Planck spectrum with temperature T. Thus if $T_{\rm d}(t_0)=T_0(1+\delta)$

$$1+\delta\simeq[1+\beta/(1-\exp-\tau_{\nu(T_0)})]^{1/4} \tag{13}$$

For $1+z_{\rm f}=200$, $T_0=2.7$, $\beta=0.25$ (for $\varepsilon=0.01$, this means $\Omega h^2=0.12$), $\tau_{\nu(T_0)}\sim1$, then $T_{\rm d}(t_0)=3.0$ K.

Assumptions about grain properties

The absorption efficiency in the IR of interstellar grains in the solar neighbourhood is not well known, so the properties of the

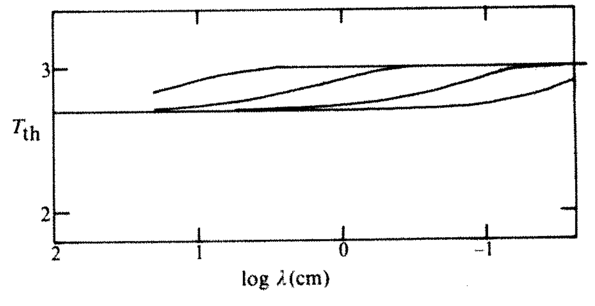

Fig. 3 $T_{\rm th}$ against λ for grains with Q_ν given by equation (14), $T_{\rm d}=3.0$, $T_0=2.7$, $1+z_{\rm f}=100$, and (from the top) $qZ=10^{-3}$, 10^{-4}, 10^{-5}, 10^{-6}.

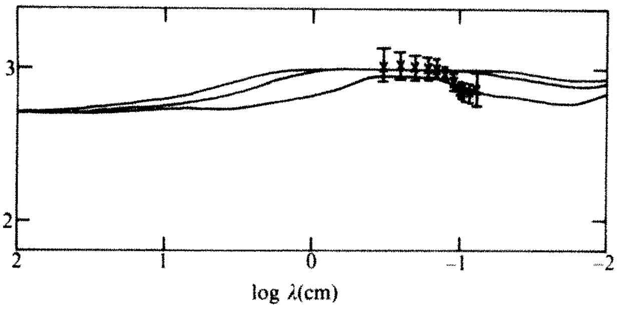

Fig. 4 T_{th} against λ for amorphous silicate grains, $T_d = 3.0$, $T_0 = 2.7$, $1 + z_f = 200$, and (from the bottom) $Z = 10^{-5}$, 5×10^{-5}, 10^{-4}, compared with Woody and Richards rates.

postulated grains in the early Universe can only be speculative. We have investigated three assumptions about Q_ν:

$$\begin{aligned} \text{(a)}\quad Q_\nu &= qx,\ x = 2\pi a/\lambda < 1/q \\ &= 1,\ x \geq 1/q,\ \text{where } q \leq 2 \end{aligned} \tag{14}$$

With $q = 2$ this represents the theoretical maximum efficiency for grains[17]. It has been argued[16] that in the far IR, grains in hot-centred molecular clouds have $q \sim 0.1$ for $a = 0.1$ μm.

(b) Amorphous silicate grains, for which we have calculated the absorption efficiency in the range 9–200 μm from the data of Day[18], used the Q_ν for terrestrial silicates given by Aannestad[19] for $\lambda < 9$ μm, and extrapolated assuming $Q_\nu \propto \nu$ for $\lambda > 200$ μm (see Fig. 1).

(c) An analytical approximation to the Q_ν for amorphous silicates, suitable for our purposes, given by

$$\begin{aligned} Q &= 0.5\,x, & \lambda &\geq 10\ \mu\text{m} \\ &= 0, & 10\ \mu\text{m} &> \lambda > 2\pi a \\ &= 1, & \lambda &< 2\pi a \end{aligned} \tag{15}$$

We have evaluated equation (10) for these types of grain with various assumptions about T_0, $T_d(t_0)$ and A (or Z). We are interested in two cases in particular: first, Rees' model[4] to explain the whole microwave background radiation from grains; and second, the spectrum observed in the millimetre and submillimetre range by Woody and Richards[20], which seems to show a significant distortion from a Planck spectrum.

We have plotted the results in the form of the thermodynamic temperature T_{th} as a function of ν, defined by $I_\nu = B_\nu(T_{\text{th}})$. Figure 2 shows models with $T_0 = 0$, $T_d(t_0) = 2.9$, for absorption efficiencies of the form of equation (14), with various values of qZ, and with $1 + z_f = 200$, compared with observations. As $q \leq 2$ (ref. 17), and in practice probably $q \leq 0.5$ (see ref. 19 and assumption (b) above), we see that the value of Z needed to fit the observations at long wavelengths is higher than that estimated by Rees[4]. We find $Z \geq 0.001$, and this exceeds the metal abundance seen in some globular clusters in our Galaxy[15]. This would imply that the matter which was to form into galaxies had to remain relatively unpolluted by the heavy elements made by Population III, an artificial situation. It is possible that some other material can be found to fill in the opacity at long wavelengths, for example, redshifted molecular lines. However, this model also fails to explain the distortion seen by Woody and Richards[20].

Figure 3 shows models with $T_0 = 2.7$, $T_d(t_0) = 3.0$, and grains of the form of equation (14) with various values of qZ, for $(1 + z_f) = 100$. Rather like the distortions caused by the effect of Compton scattering on heat input before decoupling[21], we see a gradual changeover from $T_{\text{th}} = 2.7$ at long wavelengths to $T_{\text{th}} = 3.0$ at short wavelengths. This does not correspond with what is observed.

Figure 4 shows a range of models with amorphous silicate grains and $T_0 = 2.7$, $T_d(t_0) = 3.0$, $1 + z_f = 200$. The distortions from a black-body spectrum are rather similar to those observed if $Z \sim 10^{-5}$. Figure 5 shows a range of models for grains with Q_ν of the form of equation (15) and $1 \geqq z_f = 100$, 200, 500 (the latter case is implausible by equation (8)). The analytical approximation gives results very similar to those for amorphous silicates. The crucial requirement in explaining a distortion of the type seen by Woody and Richards[20] is the sudden drop in absorption efficiency at wavelengths <10 μm. The redshifting of this drop in opacity into the millimetre range can give the observed distortions. None of the classical pre-decoupling distortions that have been predicted[21] can account for these distortions. No other grain material shows this phenomenon as strongly as silicates.

Conclusions

The Rees model comes close to fitting the whole background spectrum but seems to be in trouble at wavelengths ≥20 cm if we are restrained to plausible values of the metal abundance at early epochs. It also fails to fit the Woody and Richards[20] data, as does the classical big bang picture with or without heat input before the decoupling era.

If the Woody and Richards spectrum is taken seriously, it can be explained as being due to emission by silicate dust at early epochs. The required metal abundance is rather low, $\sim 10^{-5}$ by mass, and is consistent with limits obtained from the absence of reddening or a 2,200 Å absorption feature in the spectra of most quasars[22]. The energy input needed to distort the spectrum so drastically is substantial, requiring most of the matter in a Universe with the closure density ($\Omega = 1$) to undergo thermonuclear reactions in Population III. There are essentially no free parameters in our model because although any $z_f \geq 100$ would give the observed distortion, the grains would be melted at epochs such that $z_f \geq 200$. For the same reason the black-body spectrum that we assume to be present at epochs such that $z \gg 100$ could not be the result of some earlier thermalisation by dust (say of radiation from black holes in the cold universe of Carr[23] or the tepid universe of Carr and Rees[24]).

We have not attempted to give any astrophysical details of the proposed Population III, as these details are not likely to be observable. One obvious requirement is that the dust be spread sufficiently homogeneously to be consistent with the small-scale isotropy of the background. As the dust has a strong effect only at $\lambda \sim 1$–3 mm, this is not a very severe requirement at present. A search for small-scale structure in the millimetere background

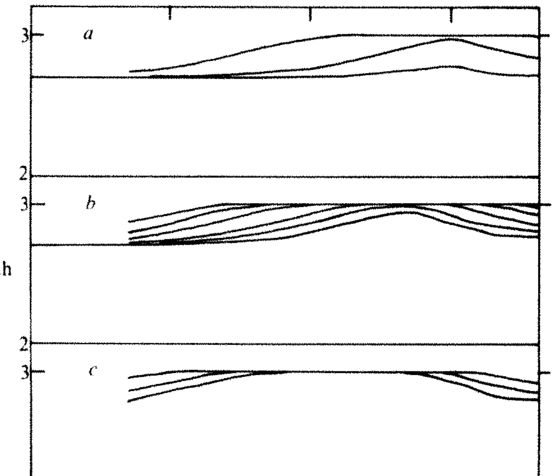

Fig. 5 T_{th} against λ for grains with Q_ν given by equation (15), $T_d = 3.0$, $T_0 = 2.7$; *a*, $1 + z_f = 100$ and (from the bottom) $qZ = 2 \times 10^{-6}$, 2×10^{-5}, 2×10^{-4}; *b*, $1 + z_f = 200$ and (from the bottom) $qZ = 5 \times 10^{-6}$, 10^{-5}, 2×10^{-5}, 5×10^{-5}, 10^{-4} and 2×10^{4}; *c*, $1 + z_f = 500$ and (from the bottom) $qZ = 10^{-5}$, 2×10^{-5}, 5×10^{-5}.

could provide an important constraint for this type of model. The ideas presented here can be tested by accurate measurements of the background spectrum both near the peak and at longer wavelengths. Improved measurements at wavelengths ≥20 cm would be a good test of models which seek to explain the whole background as thermalisation by dust or molecules (and also, incidentally, of distortion due to heat input at very early pre-recombination epochs). We are working on the exact solution of equations (5) and (6) to check assumption (9). The preliminary result is that equation (9) is valid except for redshifts very close to z_t.

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Progressive accretion in the Middle America Trench, Southern Mexico

J. Casey Moore[1], Joel S. Watkins[2], Thomas H. Shipley[3], Steven B. Bachman[4], Floyd W. Beghtel[5], Arif Butt[6], Borys M. Didyk[7], Jeremy K. Leggett[8], Neil Lundberg[1], Kenneth J. McMillen[2], Nobuaki Niitsuma[9], Les E. Shephard[10], Jean-Francois Stephan[11] & Herbert Stradner[12]

[1] Earth Sciences, University of California, Santa Cruz, California 95064
[2] Gulf Research & Development Company, PO Box 36506, Houston, Texas 77036
[3] Deep Sea Drilling Project, Scripps Institution of Oceanography, La Jolla, California 92037
[4] Department of Geological Sciences, Cornell University, Ithaca, New York 14853
[5] Phillips Research Center, Bartlesville OK 74004
[6] Institut und Museum fur Geologie und Paleontologie, Universitat Tubingen, Tubingen, FRG
[7] Research and Development Laboratory, Empresa Nacional del Petroleo, Concon, Chile
[8] Department of Geology, Imperial College of Science and Technology, London SW7, UK
[9] Institute of Geosciences, Shizuoka University, Shizuoka, Japan
[10] Department of Oceanography, Texas A & M University, College Station, Texas 77843
[11] Department de Geotectonique, Universite Pierre et Marie Curie, Paris, France
[12] Geologische Bundesanstalt, Postfach 154, Rasumofskygasse 23, A-031 Vienna, Austria

The lithology and age distribution of a seismic unit characterised by landward dipping reflectors suggest progressive underthrusting and uplift of trench deposits along the inner slope of the Middle America Trench. The fastest uplift and deformation rates, and the most rapid change in tilt of dipping reflectors coincide near the base of the trench slope and diminish rapidly landwards.

PLATE tectonic theory as well as observations at convergent margins have produced several models of trench slopes each making specific predictions as to the origin, age sequence, and structural configuration of the rocks incorporated therein[1–8]. Off-scraped deep-sea rocks arranged in an imbricate stack of landward dipping thrust slices constitute a popular trench–slope model[1,2] which has been applied widely to both modern subduction zones and their supposed ancient equivalents. This imbricate thrusting model is based primarily on seismic reflection data[2] but is poorly substantiated by drilling.

Thick slope aprons and deep water have hindered sampling of inferred off-scraped deposits by drilling in the Marianas and Japan trenches[6,7,9]. Recovery of inferred off-scraped deposits elsewhere has been limited to restricted areas along a given trench slope and has not provided the data necessary to constrain models of trench–slope evolution[10–12]. Off southern Mexico the lower slope of the Middle America Trench is covered by a thin slope apron overlying a zone of landward dipping reflectors[13] similar to those which elsewhere are considered to constitute off-scraped deep-sea deposits. In this area, the shallow trench and a thin slope apron permitted drilling of three holes into the zone of landward dipping reflectors which provided substantial data on trench–slope evolution. The drilling was conducted during Leg 66 of the Deep Sea Drilling Project (DSDP).

In the Leg 66 area (Fig. 1) the Middle America Trench is characterised by a narrow shelf, steep inner slope and no forearc basin. Our drilling results confirm previous magnetic[14] and seismic reflection[13] studies indicating that the Mesozoic to Precambrian crystalline basement approaches within 35 km of the trench axis which severely limits the width and volume of off-scraped material. This limited accretionary zone probably developed during the Neogene synchronously with magmatism in the Trans-Mexican belt to the north-east. A more extensive Mesozoic and Palaeogene forearc and accreted zone may have been previously removed[14].

Leg 66 drilling results

On Leg 66 DSDP we drilled eight sites (Fig. 1) five of which (Sites 486–488, 491, and 492) are specifically relevant to trench–slope models.

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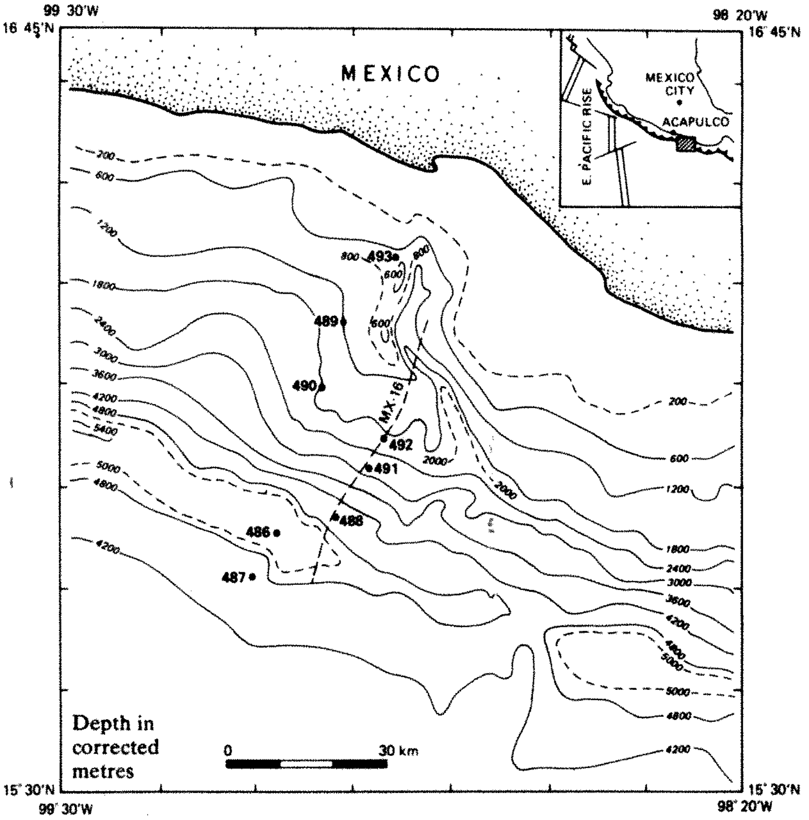

Fig. 1 Location of Leg 66 drilling sites. Dashed line covers the extent of the seismic profile MX16 shown in Fig. 3. Note the prominent submarine canyon which provides a conduit for transporting coarse-grained detritus directly into the trench and bypassing the slope on which Sites 488, 491 and 492 were drilled.

Sites 486 and 487 were located in the trench and on its outer slope respectively. These sites penetrated trench deposits and sediments overlying oceanic crust thereby sampling the type of material entering the subduction zone and providing a basis for interpreting deposits cored from the trench slope. At site 487 seaward of the trench, we penetrated 115 m of Pleistocene and latest Pliocene hemipelagic mud overlying 55 m of Pliocene to late Miocene brown clay, bottoming in basalt. At Site 486 two holes were drilled on the trench floor separated by ~1 km. In each case the abundance of sand caused the hole to collapse. The thickest section cored at Site 486 consisted of 38 m of fine to very coarse sand with mud. Piston cores taken by the University of Texas Marine Sciences Institute (UTMSI) elsewhere in the trench recovered abundant sand[15] suggesting that this is the dominant lithology in at least the uppermost deposits of the modern trench. Conversely UTMSI cores from the slope apron and lower slope basins in the Leg 66 area contain mud with rare fine thin sand and silt beds[15]. The high content of quartz and feldspar in the sand from the trench suggests it was derived from the crystalline basement of the adjacent continental margin probably via a prominent submarine canyon[16] (Fig. 1). Although the sand in the cores at Site 486 is massive, diagnostic sedimentary structures in some of the UTMSI piston cores from the trench indicates deposition by turbidity currents. Apparently the coarse sand cored at Site 486 moved across the narrow continental shelf and down the submarine canyon to the trench as a density underflow and effectively bypassed the slope.

Sites 488, 491, and 492 penetrated the lower to mid-slope region along UTMSI seismic line MX-16 (Fig. 1) and provided critical evidence for evaluating trench-slope evolution. Site 488, located at the toe of the trench slope at a water depth of 4,254 m, penetrated 313 m of Quaternary mud which below 170 m sub-bottom contains local thin silt and muddy sand beds (Fig. 2). Underlying the mud we cored 115 m of Quarternary mudstone and sand to pebbly sand bottoming at 428 m below the sea floor.

The sand-bearing unit of Site 488 must have been deposited in a major turbidite channel or basin. This unit could have accumulated in a turbidite channel or basin on the slope, however, such a structure was not detected in an exhaustive site survey and would have to have been obliterated. In view of the similarity of sand-bearing unit to deposits cored in the trench, we prefer to interpret it as an uplifted trench sequence. The virtual absence of significant sand beds in the upper Quaternary slope and slope–basin sediments from the piston and drill cores in the Leg 66 area, and the probable topographic diversion of down-slope sand transport from the site (Fig. 1) both argue against deposition of abundant coarse sand on the slope. If the sand-bearing unit accumulated in a trench of comparable depth to the present trench, then these sediments have been uplifted at a rate of 400–500 m Myr^{-1}.

The uniform hemipelagic muds above 170 m at Site 488 probably were deposited near their present position on the lower slope. The thin silt and sand beds between 170 and 313 m require a nearby conduit intermittently supplying fine sand and silt. These thin silt and sand beds probably accumulated when the underlying sand and mudstone unit was slightly elevated above the trench floor but still receiving fine-grained turbidites from the upper portions of large density underflows in the trench axis.

Site 491 lies at a water depth of 2,883 m, ~2 km above and 14 km landward of the Middle America Trench (Figs 1 and 3). Here, we cored 58 m of mud, overlying 380 m of mud containing local thin, fine sand layers, on top of 104 m of sand and pebbly sand interbedded with mudstone of early Pliocene age (Fig. 2). Below 39 m sub-bottom (~2 Myr BP) the sediments are barren of planktonic foraminifera and the nannofossil assemblages are much less abundant suggesting the site passed through the calcite compensation depth (CCD) at this time. In the Leg 66 area surface samples from piston cores and the DSDP sites place the modern CCD at 3,000–3,500 m below sea level. The CCD at 2 Myr BP is placed at 2,750–3,250 m with the shallowing in depth inferred from the documented time-depth variations in the central Pacific Ocean[17]. The depth of the CCD at Site 492 for 4 Myr BP is estimated similarly. We interpret the depositional environments of Site 491 as at Site 488, in terms of the modern example; the sand, pebbly sand and mudstone probably represent trench deposits and the overlying fine-grained sands, silts and muds probably accumulated on the slope during uplift of the underlying sediments. Alternatively, but less likely, the sand, pebbly sand, and mudstone could have accumulated in a lower slope basin or turbidite channel. If the sand, pebbly sand and mudstone accumulated in the trench or on the lowermost slope at water depths of 4–5 km it was uplifted initially at a rate of about 400 m Myr^{-1}. The uplift rate apparently slowed to less than 100 m Myr^{-1} after 2 Myr BP when the site passed through the local CCD at a depth of about 3,000 m.

At Site 492 in a water depth of 1,942 m we penetrated 247 m of Quaternary to Upper Miocene mud and mudstone overlying 43 m of Upper Miocene mudstone with sand and granular sand. In sediments about 4 Myr old at 50 m subbottom an abrupt decrease in carbonate content down-hole suggests the site passed through the CCD which is estimated to have been between 2,500 and 3,000 m below sea level at this time. Below 120 m the sediments are barren of foraminifera and contain a *Teichichnus–Zoophycos–Chrondrites* trace fossil assemblage suggesting accumulation at depths of 4 km or greater[18]. The sand bearing unit was probably deposited in the trench, based on the lines of reasoning for similar sand sequences at Sites 488 and 491. The age and depth of origin of the trace fossil assemblages, and the time of passage of Site 492 through the CCD define an initial uplift rate of about 500 m Myr^{-1} decreasing to about 200 m Myr^{-1} after 4 Myr BP.

Sporadic occurrences of shallowly dipping beds usually constitute the initial downhole evidence of deformation at Leg 66 drilling sites. At greater depths a gradual transition to cores with repeated occurrences of dips >25° represents the threshold of what we define as significant deformation. In addition to tilted bedding, discrete inclined fractures were common and small scale folds and overturned beds occurred locally. At Site 488 sediments between 210 and 248 m are characterised by healed fractures that truncate bedding and produce zones of stratal discontinuity, evidenced by discontinuous silt lenses in a mud matrix. At Site 492 mudstone intervals below 250 m resemble scaley clay where they are cut by slickensided anastomosing fractures. Deformation begins at shallower depths below the modern sea floor but in older sediments successively landward through Sites 488, 491 and 492 (Fig. 2); the age of the threshold of significant deformation is 0.6, 3.0 and 5.8 Myr at Sites 488, 491 and 492 respectively. Slope sediments are involved in the deformation in all cases; at Site 492 sediments that accumulated at depths as shallow as 3–4 km are deformed significantly.

Seismic reflection data

An extensive grid of multichannel seismic reflection profiles in the Leg 66 area facilitated site selection and aided interpretation[13]. Figure 3 is a migrated depth-section through Sites 488, 491 and 492, and shows the oceanic basement reflector extending about 15 km beneath the trench slope. Reflections defining the trench fill seem to be acoustically transitional to the zone of landward dipping reflectors underlying the trench slope.

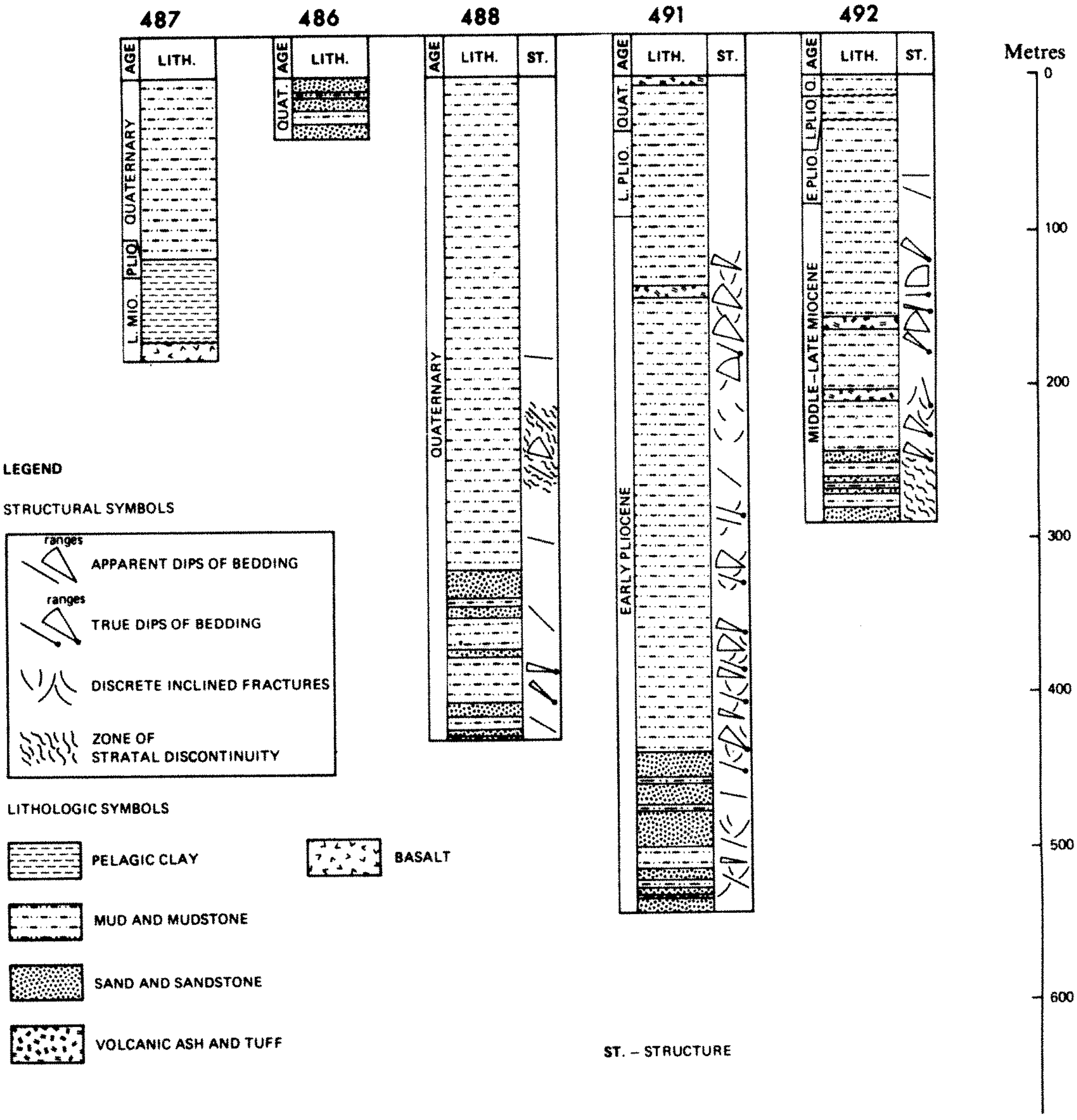

Fig. 2 Summary of lithology and structural geology for Sites 486–488, 491 and 492. The sand-bearing units, encountered at 313, 438, and 247 m at sites 488, 491 and 492 respectively, correlate with the zone of landward dipping reflectors whereas the overlying mud-rich units correlate with the slope apron (Fig. 3). The depth of significant deformation (repeated occurrences of dips greater than 25° occurs at 220, 116, and 80 m at Sites 488, 491 and 492 respectively.

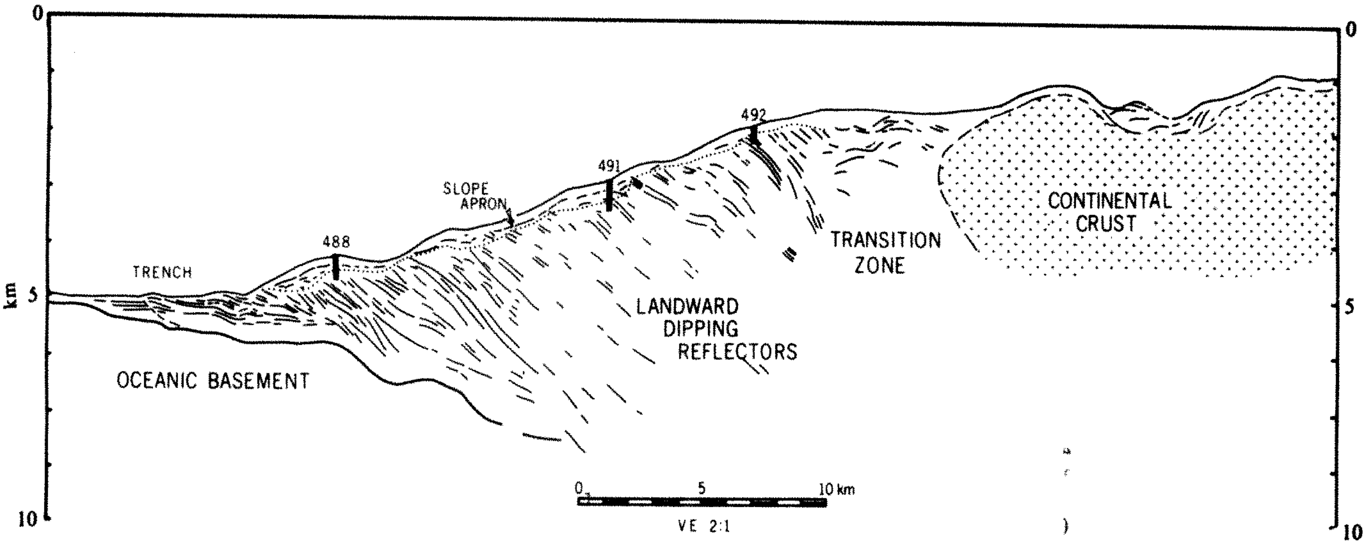

Fig. 3 Line drawing of the depth section MX16. Dotted line separates the slope apron from the landward dipping reflectors. The gas hydrate reflector is omitted for clarity.

Progressive landward inclination of dipping reflectors begins in the lowermost slope area; upslope their inclination remains relatively uniform. The zone of dipping reflectors is overlain by a mantle of discontinuous and indistinct reflectors, the slope apron, with a maximum thickness of 500 m. This slope apron consists of reflectors parallel to both the slope and to the underlying landward dipping reflectors.

The mud-rich sediments comprising the upper portion of the section at Sites 488, 491 and 492, the inferred slope deposits, correlate with the mantle of discontinuous and indistinct reflectors. The zone of landward dipping reflectors correlates with interbedded sand and mudstone units which begin at depths of 313, 438 and 247 m respectively at Sites 488, 491 and 492. Thus many of the landward dipping reflectors here are probably generated not by faults but changes in physical properties across bedding surfaces between the sand and mudstone units. The acoustic similarity of the trench fill and the landward dipping reflectors[13] also suggests that the latter represent bedding surfaces and that the interpretation of the interbedded sands and mudstones as trench deposits is correct.

Conclusions and discussion

The landward increase in age of the zone of landward dipping reflectors constitutes one of the most fundamental results of drilling on Leg 66 (Fig. 4). Such a systematic age progression indicates seaward growth of the continental margin irrespective of how the dipping reflectors are interpreted. We believe that these landward dipping reflectors are bedded units generated by thick sand and mudstone sequences which probably accumulated in the trench, or less likely on the lower slope. Thus, the landward increase in age in these dipping reflectors represents a stratigraphic inversion most simply explained by underthrusting along surfaces sub-parallel to bedding.

Shipboard palaeontological results did not resolve stratigraphic inversions in a single drillhole, even though one site (Site 488) was located to penetrate a seismically inferred thrust fault. Nevertheless, zones of stratal discontinuity and scaley clay, and anomalies in physical properties, suggest faulting and/or shear zones cutting the section cored at Sites 488 and 492. The gross stratigraphic inversion between sites suggests that thrusting occurs within discrete zones between our drilling locations and/or as distributed shear on numerous faults of small displacement. The pervasively sheared and disrupted fabrics common to inferred ancient and subduction complexes support the latter interpretation.

Although our palaeobathymetric data are preliminary, they consistently indicate uplift of the trench slope at Sites 488, 491 and 492. Quaternary rates are most confidently established suggesting uplift of 400–500 m Myr^{-1} at Site 488, decreasing to 200 m Myr^{-1} or less at Sites 491 and 492. Significantly, palaeobathymetric data suggest that the inferred trench and/or lowermost slope deposits of Sites 491 and 492 underwent rapid uplift which subsequently decreased to the slower rates characteristic of the Quaternary. Thus both the present distribution of Quaternary uplift rates and the historical records of Sites 491 and 492 argue for a high rate of uplift on the lowermost slope with a diminishing rate of uplift landwards.

The deformation in cores from Sites 488, 491 and 492 includes overturned folds, small-scale disruption of layering and scaley clay all of which occur commonly in subaerially exposed subduction complexes. In the Leg 66 cores the trench and the immediately overlying lowermost slope sediments are similarly deformed though the intensity of deformation decreases upsection through sediments accumulated at shallower depths. The tilting of sedimentary layering in cores accumulated at 3–4 km of water depth (50–120 m sub-bottom) at Site 492 indicates continuing deformation of the slope a substantial distance from the trench. Thus since the deformation is

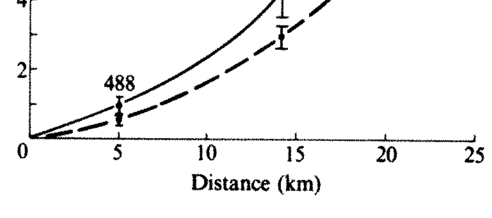

Fig. 4 Age of dipping reflectors (solid line) and youngest significant deformation (dashed line) versus distance from base of trench slope. Age of dipping reflectors taken from age of top of sand-bearing units (Fig. 2). Age at which youngest significant deformation occurs specified in caption, Fig. 2. Error bars indicate uncertainty in biostratigraphic age determinations.

apparently continuing upslope, away from the trench, the age of significant deformation at each site (Fig. 4) can be used as an indicator of the relative rates of deformation. The time required to develop significant deformation increases by a factor of 5 from 5 to 14 km from the base of the slope, and by an order of magnitude from 5 to 20 km from the base of the slope (Fig. 4). In other words, the rates of deformation at Sites 491 and 492 are respectively one-fifth and one-tenth of that observed at Site 488.

The folded beds and disrupted layering documented at Sites 488, 491 and 492 are probably due to both tectonic and gravity processes; the evidence concerning individual structural features is ambiguous. Nevertheless, correlation of the high rate of deformation at Site 488 with the high rate of uplift and rapid change in tilt of dipping reflectors indicates a concentration of tectonism near the base of the trench slope. Thus if gravitational processes are active in producing the structural features seen in the cores they too are accentuated at the base of the trench slope probably in response to the concentration of tectonic activity.

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A volcanic ash generated by explosions where ignimbrite entered the sea

George P. L. Walker

Geology Department, University of Auckland, Private Bag, Auckland, New Zealand

A remarkably widely dispersed and crystal-rich volcanic ash-fall deposit is described, which resulted from immense explosions generated in the littoral zone in New Zealand where a major rhyolitic ignimbrite flowed into the sea. This partially explains what happens when large pyroclastic flows enter water.

THE rhyolitic volcanoes of the Taupo Zone have produced extensive air-fall ashes in addition to the ignimbrites for which they are better known. The tephrochronology of these ashes is well documented[1–4], but volcanological interpretations have seldom been attempted. The greatest air-fall deposit of the past ~50,000 yr is the Rotoehu ash, part of which is described and interpreted here.

The Rotoehu ash is remarkable for its large volume, previously estimated[5] to be at least 50 km^3 but now believed to be much more. It is also widely dispersed over most of the North Island of New Zealand, and is extremely rich in crystals which show up in its coarser beds. An isopach map is given by Vucetich and Pullar[5]. It is a distinctively shower-bedded fall deposit, and here we consider the thickest and uppermost and most widely dispersed of the three coarsest beds (bed G in Fig. 2).

The Rotoehu ash is intimately associated with the non-welded Rotoiti ignimbrite, the latest major ignimbrite from the Okataina volcano, and Nairn[6] has demonstrated that parts of the ash underlie, overlie, and are interbedded with flow units of the ignimbrite. The ignimbrite covers about 830 km^2, Fig. 1, and is known by its stratigraphic name as the Rotoiti Breccia. It is believed to have originated from a vent or vents in the Haroharo complex of Okataina volcano, and reached the coast of the Bay of Plenty 30 km from source along a front of more than 10 km. The ^{14}C age is about 42,000 years[7,8]. The estimated volume is 50 km^3.

Characteristics of the deposit

Bed G has a loose and sandy texture, and within 50 km of the Rotoiti ignimbrite outcrop it contains scattered fragments of ragged pumice up to several centimetres in size. It is conspicuously rich in crystals, resembling a waterlain sand. The relative uniformity in grain-size and thickness over a great area, the perfect mantle bedding, and the lack of rounding of the grains,

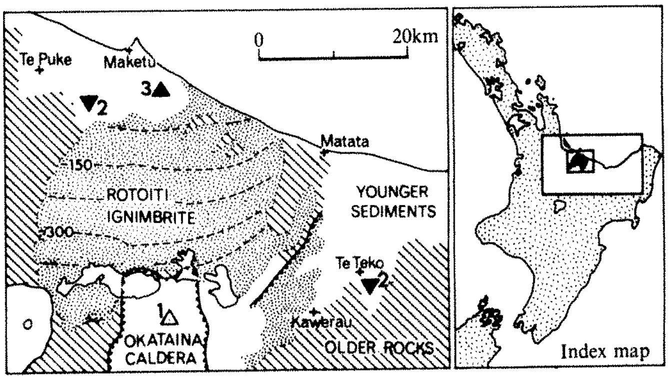

Fig. 1 Map showing the main outcrop of the Rotoiti ignimbrite, and the approximate locations of its source vent (1), the two littoral vents of Rotoehu ash bed G (2), and the littoral vent of ash bed E (3). The ignimbrite forms a northward-sloping aggradational surface (dashed lines indicate generalised contours, heights in metres); anomalously high south of Matata which is attributed to post-Rotoiti uplift of the land bordering the young rift vally in which Te Teko is situated. An older non-welded ignimbrite separated from the Rotoiti by a soil also occurs but is not distinguished on the map. The extent of the Rotoiti ignimbrite under the sediment flats of Te Puke and Te Teko is not known. The index map of the North Island of New Zealand shows the location of the studied area.

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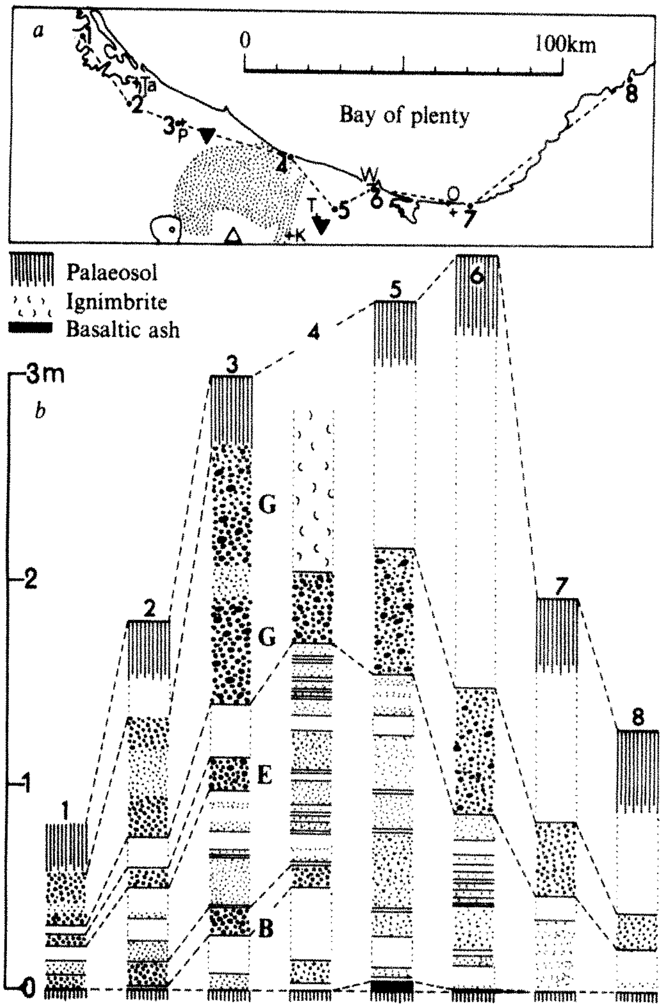

Fig. 2 *b*, Profiles of the Rotoehu ash at locations shown on the sketch map (*a*), in which the grain size of the beds is shown by the coarseness of the stipple (the finest ash is unstippled), showing the relative positions and thickness variations in the three main sandy beds, B, E and G. Place names K, Kawerau; O, Opotiki; P, Te Puke; Ta, Tauranga; T, Te Teko; W, Whakatane.

however, leave no doubt as to its shower origin. In the east and south, G is the coarsest and most prominent sandy bed in the Rotoehu ash. It is underlain by well bedded fine and sandy ashes, and is overlain by fine loess-like ash. In the west, G is the thickest, though not always the coarsest, of the sandy beds; it has a rather finer middle part and grades upwards to finer ash, and the weathered zone often extends well down into it, destroying its loose sandy texture.

The maps of bed G, Fig. 3, reveal the wide dispersal; the thickness and maximum grain-sizes decrease to half their value over a distance of 20–50 km, which is a remarkably low decrease rate for a fall deposit.

Sieve analyses have been made of 54 samples of G, using a set of sieves in which the aperture size of each is half that of the sieve above, and for each sample the percentages of components (pumice, free crystals, and lithics) have been determined for each size class down to 0.25 mm. For convenience, the size class is denoted by the aperture size of the sieve that retains it. 'Pumice' includes vesiculated juvenile fragments whatever their size, and also the bubble-wall shards which are abundant in fractions finer than 0.25 mm.

Lateral variations in grain-size characteristics are shown in Fig. 4*a*, and in crystal content by Figs 4*b* and 5. Figure 4*c* shows that the percentage of crystals decreases outwards in each size class due to aeolian fractionation: crystals have fallen closer to source than pumice of the same size. The decrease rate is remarkably low: crystals constitute more than 50% of the 0.5 mm class out to as far as 80 km from the ignimbrite outcrop.

South of Rotorua, G is missing and ash rich in accretionary lapilli occurs instead. Fine ash was thought to be flushed by rain from the eruptive cloud together with coarser material near Rotorua so that bed G is not recognisable as such, and by the time the cloud spread farther south it had largely been cleansed of ash.

Origin

The thickness and maximum grain-size decrease rate resembles that of a plinian pumice, but bed G differs from such a deposit in three ways. First, it totally lacks ballistic clasts; the largest pumice and lithic fragments noted measure 8.4 and 4.6 cm in maximum size respectively. Second, the median diameter is never coarser than 1 mm. Third, the overall grain-size distribution clearly has a strong mode in the 0.5 and 0.25 mm classes which predominate in all samples out to 100 km from the ignimbrite, Fig. 4*a*, and this mode consists mainly of crystals.

Bed G is interpreted as originating from vast explosions at two places where pumice flows of the Rotoiti ignimbrite entered the sea, based on two independent lines of evidence: the field data, and the peculiar grain-size characteristics of the bed.

First, the thickness and maximum grain-sizes reach a maximum at two places about 45 km apart, one near Te Puke and the other near Te Teko, Fig. 3 *a,c,d*, and likewise the content of coarse material from sieve analyses, Fig. 3*e*. These sites are on alluvium and are believed to mark the rootless vents of the littoral explosions by which bed G was generated. Sea bays are postulated to have projected to them at the time of the eruption. The thickness and grain-size decrease rate out from the Te Teko vent is lower than from the Te Puke vent suggesting that the Te Teko explosion was the more powerful, and the lack of stratification in bed G in the eastern area indicates a single short explosion of uniform intensity.

Second, G originated from a pyroclastic mixture that was more like that of a pyroclastic flow than that ejected at a primary explosion vent. Consider the special grain-size features of ignimbrite and their origin. Ignimbrite is constituted from the pyroclastic mixture which leaves the primary vent. The crystals are phenocrysts liberated from the magma, and the phenocryst content of large pumice clasts taken from the ignimbrite is believed to equal that in the original magma and for the Rotoiti ignimbrite and bed G is 24 wt %.

Three processes then modify the pyroclastic mixture so that the resulting ignimbrite departs considerably from the initial population. One is a loss of fine vitric particles, from the eruptive column and the moving flow, which eventually settle as a co-ignimbrite ash-fall largely beyond the limits of the ignimbrite. The loss generally amounts to about half of the total erupted mass, as is indicated by the two-fold concentration of free crystals in the ignimbrite[9,10]. The second process is the settling out of heavy clasts from the pyroclastic flow, the largest as a near-vent lag-fall deposit[11], and smaller ones as a lithic concentration zone near the base[12], or as a ground layer beneath, the ignimbrite[13]. This results in an outward decrease in maximum lithic size and content[14]. The third process is a progressive fragmentation of pumice within the flow.

All three processes seem to have operated in the Rotoiti ignimbrite. In distal exposures the ignimbrite has a low content of lithics and a maximum lithic size of ~5 cm; the content of free crystals is high, with a strong mode in the 0.5 and 0.25 mm classes; and the pumice distribution is strongly fine-skewed.

The characteristics of the distal Rotoiti ignimbrite are summarised by Fig. 6*a*. A big contrast exists between samples taken from near the base or from the middle/upper part, the former being much richer in lithics and the latter in coarse pumice (Fig. 5), and Fig. 6*a* is averaged from three samples taken from each position. The ignimbrite consists of many flow units, and the one which gave rise to bed G has not been identified.

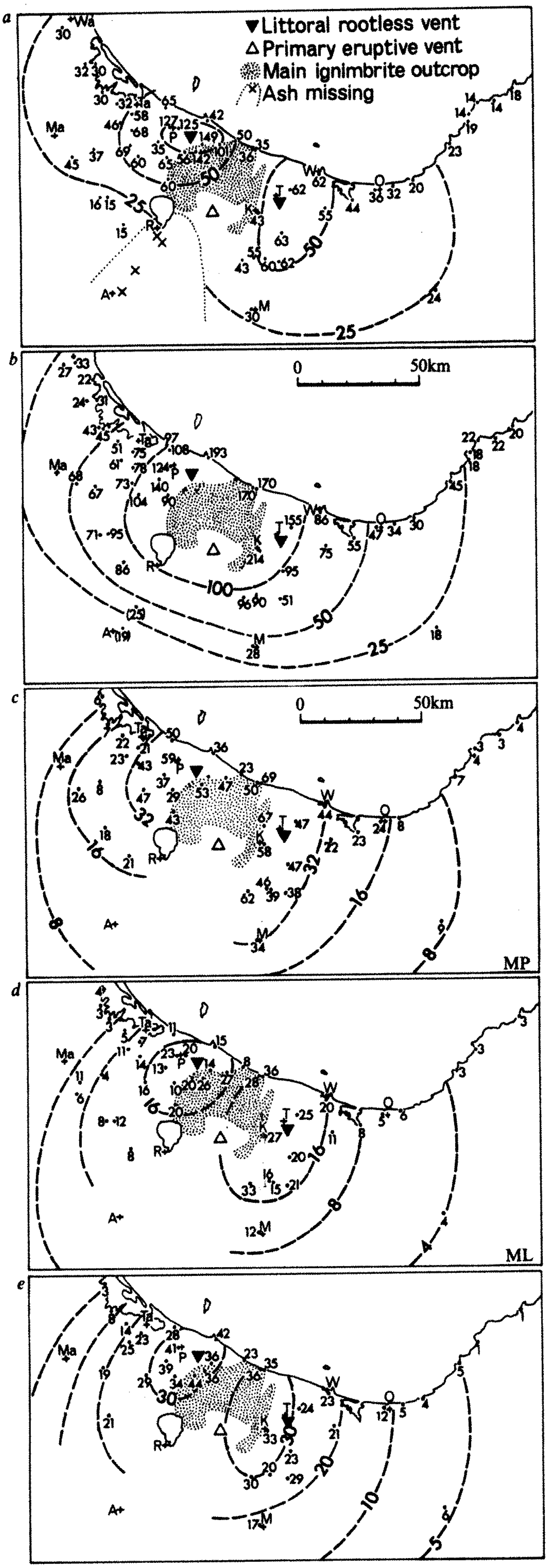

Fig. 3 Dispersal maps of the Rotoehu ash bed G. ▼, The rootless vents of Te Puke (P) and Te Teko (T). *a*, Thickness (cm); *b*, thickness of the sub-G part of the Rotoehu ash (cm); *c*, average maximum diameter of the three largest pumice clasts (mm); *d*, average maximum diameter of the three largest lithic clasts (mm); *e*, wt % of material coarser than 1 mm. Place names as Fig. 2, with *A*, Atiamuri; Ma, Matamata; M, Murupara; R, Rotorua; Wa, Waihi.

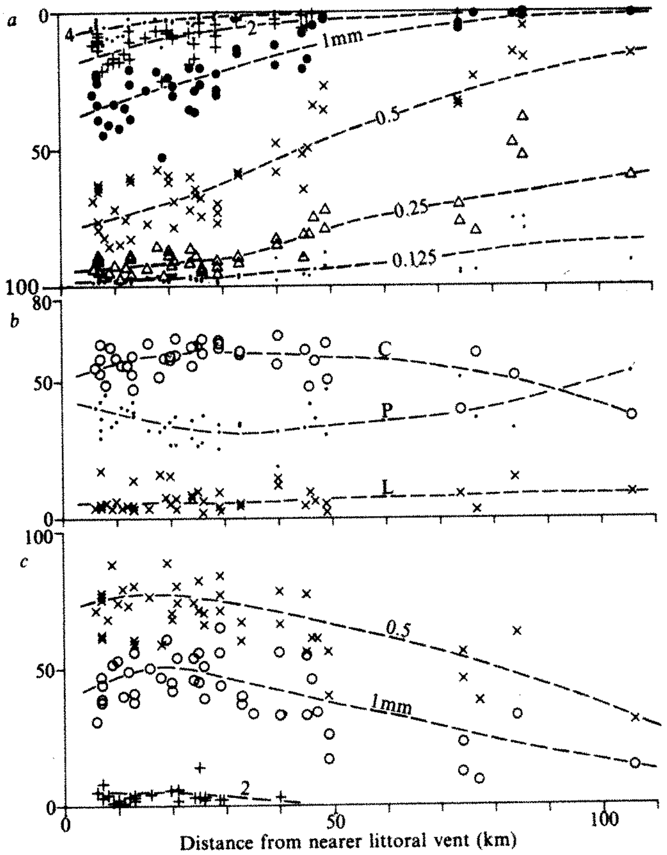

Fig. 4 Characteristics of the Rotoehu ash bed G as they vary with distance from the nearer littoral vent: *a* Cumulative wt% coarser than the size stated, for all sieved samples. Note the broadness of the band between 1 and 0.25 mm; the 0.5 and 0.25 mm size classes predominate in all samples irrespective of their distance from the vent. *b*, wt% of pumice (P), crystals (C) and lithics (L) in the sum of the 0.25 and coarser classes, recalculated to 100% total. Note that crystals predominate in almost all samples; *c*, The wt% of crystals in three grain-size classes, expressed as a percentage of that class.

The overall grain-size constitution of G has been partially determined as for the Taupo plinian pumice[15], by drawing maps of the mass per cm^2 of each size class and then integrating to yield the total mass of the class. These integrated values are plotted in Fig. 6*b* and compare closely with the average Rotoiti ignimbrite. The origin of bed G from Rotoiti ignimbrite material can be deduced from the size data and constitution since these are predetermined by the pyroclastic mixture available, characterised by a small maximum clast size, a high content of crystals, and a small content of pumice coarser than 1 mm.

Phreatomagmatic pyroclastic deposits formed where water enters the primary vent have a similar strongly fine-skewed population[16], but ballistic fragments are also invariably present. Furthermore, such eruptions are pulsatory, so that fine material from weaker activity falls together with coarse from stronger activity, to produce poorly sorted deposits even very close to vent; in contrast, bed G is well sorted everywhere.

The volume of bed G within the 25 cm isopach is 6 km^3, assuming bilateral symmetry about the Te Puke–Te Teko line, but much lies outside this isopach. When a plot of the area enclosed by isopachs is extrapolated out to 1 cm, the volume by integration is 20 km^3, but the extrapolation is uncertain. The formula of Cole and Stephenson[17,18] (taking the two vents separately) yields a total of 14 km^3.

Another way of estimating the volume is by assuming that G originated from the average Rotoiti ignimbrite of Fig. 6*a*. Based on the quantity of material 0.5 mm or coarser (Fig. 6*b*), the total mass of G is 12×10^{15} g (equals 11 km^3 at a density of 1.07 g cm^{-3}); based on the content of crystals 0.5 mm or coarser, the mass is 18.6×10^{15} g (= 17 km^3); and based on the quantity of crystals in the 0.5 mm class the mass is 20.5×10^{15} g(= 19 km^3).

Discussion

The exceptionally wide dispersal of bed G, and the efficient aeolian fractionation of crystals even 100 km from source, indicate that the secondary eruptive columns above the littoral explosions must have been exceedingly powerful. The area enclosed by selected maximum pumice and maximum lithic size isopleths (Table 1) is comparable with that given by the most powerful known plinian eruption, that of Taupo AD 131 (ref. 15). It is very much greater than for other known deposits of rootless vents (for example, in Iceland[19] and Hawaii[20]).

The extreme violence of the Rotoehu event must result from an exceedingly high energy release rate. The best mechanism seems to be one in which about 15 km^3 of hot pyroclastic flow entered the sea very rapidly at Te Puke and Te Teko and spread over perhaps tens of square kilometres before coming to rest. The first part to enter the sea became well mixed with water, and the rapid exposure of a great surface area of the hot particulate material resulted in a very rapid heat exchange with the water. This part, however, was rapidly over-ridden and buried beneath newly-arrived material, delaying the explosive expansion of the resulting steam. When the supply of material fell, the pressure build-up exceeded the rate of addition of material, and explosive disruption of the entire mass then occurred.

The abnormally high crystal content of bed G stems from an initial high magmatic content, combined with the subsequent successive operation of two crystal concentration processes: the selective loss of vitric dust above the primary vent and moving flow, and the aeolian fractionation of material falling from the littoral explosions. The exceptional violence of the explosion resulted in a high crystal content persisting even to 100 km from source.

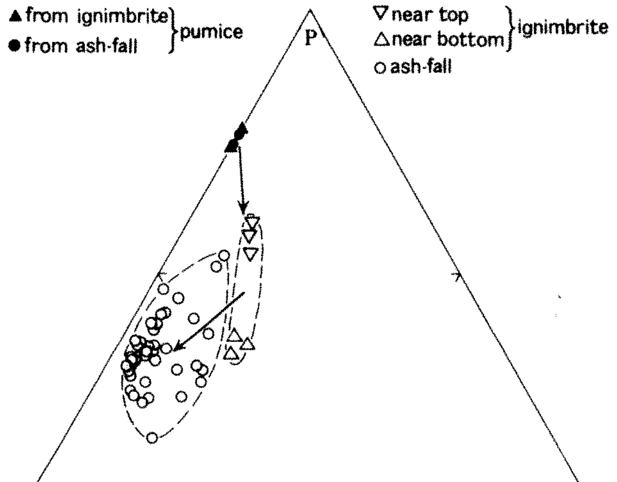

Fig. 5 Triangular plot showing the weight percentages of pumice (P), crystals (C) and lithics (L) in samples of the Rotoehu G ash and Rotoiti ignimbrite, for the sum of the 0.25 mm and coarser classes recalculated to 100% total. Crystal contents of pumice clasts from the ash and ignimbrite were determined by crushing followed by mechanical separation, and are thought to be representative of the original magma. The arrows show the two stages of crystal enrichment beginning with the original porphyritic magma.

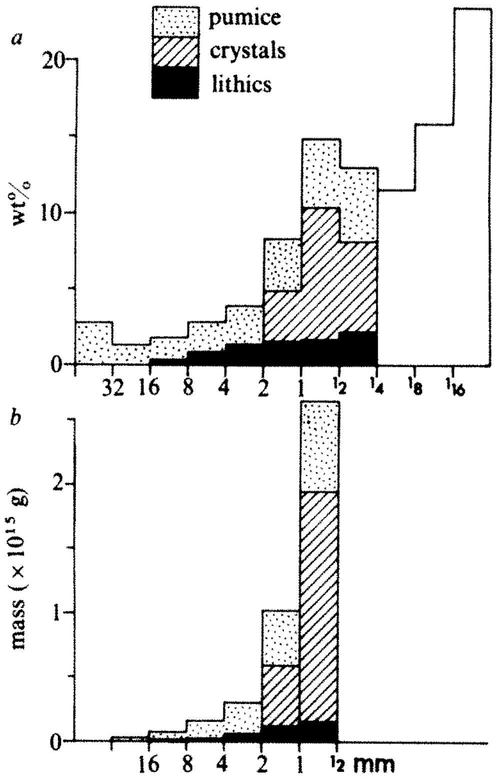

Fig. 6 *a*, Histogram of the average grain-size distribution in six sieved samples of the Rotoiti ignimbrite; the bars are subdivided on their weight percentages of components. *b*, Histogram of the total mass of grain-size classes and their components in the mapped part of the Rotoehu ash bed G; the vertical scale has been adjusted so that the total length of bars for the 0.5 mm and coarser classes in (*a*) and (*b*) is the same.

The other two main sandy layers (B and E) in the Rotoehu ash have similar characteristics and formed by similar explosions of earlier Rotoiti ignimbrite flow units. Bed E was evidently formed where a flow entered the sea south-east of Maketu (3, Fig. 1). Its volume and dispersal are comparable with the eastern part of G from the Te Teko rootless vent. The fine ashes within the Rotoehu ash may be normal co-ignimbrite ash-falls or may represent weaker littoral activity.

The Te Teko explosion which gave rise to half of bed G and that which gave rise to bed E seem to have been the most violent volcanic explosions yet documented by ash dispersal studies. This is reminiscent of the 1883 eruption of Krakatau, which was accompanied by major explosions and culminated in three immense paroxysms during which tsunamis were generated. It has long been supposed that seawater was a major factor in producing such extreme violence, but there has always been uncertainty regarding the mechanism: whether it was by the sea entering the magma chamber or not.

Another possibility is that the Krakatau explosions were indeed caused by the interaction of magma and the sea, but the explosions were littoral ones where pyroclastic flows entered the water as at Te Puke and Te Teko. The classic studies of the pyroclastic products of Krakatau by Verbeek[21] and Stehn[22] indicate that puroclastic flow deposits on land were formed at the time of the biggest explosions. Testing of this idea, must, however, await a critical re-examination of the pyroclastic products of this event.

The picture which emerges is that ash bed G was deposited from an immense mushroom cloud which was tens of kilometres high and rapidly expanded until it was well over 200 km in diameter. This mushroom spread laterally outwards nearly

Table 1 Areas enclosed by maximum grain size isopleths ($\times 10^3$ km^2)

	Maximum pumice (mm)				
	64	32	16	8	4
Rotoehu bed G*	0.2	3.4	14	24	44
Taupo plinian	5	9.9	18	28†	40†
Pompei plinian[23]	0.3	0.9	1.9†	3†	—
	Maximum lithic (mm)				
	32	16	8	4	
Rotoehu bed G*	0.1	2.5	12	25	
Taupo plinian	2.3	4.2	7	15	
Pompei plinian[23]	0.6	1.1†	1.6†	—	

* Assuming a symmetrical distribution on either side of the Te Puke–Te Teko line.

† Extrapolated values.

uniformly in all directions. The material which fell 100 km out from source has a median terminal fall velocity of 3 m s^{-1}, for which the fall time from a height of 10–50 km in normal atmosphere is 35–70 minutes. This suggests that the outward radial velocity of the expanding cloud averaged 180–90 km h^{-1} neglecting the dustiness of the atmosphere at the time and turbulence. The lateral expansion was apparently much greater than is normal in volcanic explosions, and was comparable with the most powerful one currently known. In normal explosions, the expansion is due to magmatic gases which constitute a few wt% of the magma. In the Rotoehu case it is possible that steam constituted a few tens of wt% of magma involved.

This ash is an example of a new class of pyroclastic deposit for which the name 'littoral co-ignimbrite ash-fall' is proposed.

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Nucleotide sequence of the hepatitis B virus genome (subtype ayw) cloned in *E. coli*

**Francis Galibert*†, Elisabeth Mandart*, Françoise Fitoussi*,
Pierre Tiollais‡ & Patrick Charnay*§**

* Laboratoire d'Hématologie Expérimentale, Centre Hayem, Hôpital Saint-Louis, Paris, France
‡ Recombinaison et Expression Génétique (INSERM U.163), Unité de Génie Génétique, Institut Pasteur, Paris, France

The complete nucleotide sequence of hepatitis B virus genome (subtype ayw) cloned in Escherichia coli *has been determined using the Maxam and Gilbert method and the dideoxynucleotide method. This sequence is 3,182 nucleotides long. Location of the nonsense codons shows that the coding capacity of the L chain is larger than the coding capacity of the S chain. Eight open regions, able to code for polypeptide chains larger than 100 amino acids, have been located. Region 6, which is the largest, covers more than 80% of the genome. The gene* S *which codes for polypeptide I of the Hbs Ag and was previously located between coordinates* 95.1 *and* 73.6 *is contained in region* 7.

HEPATITIS B VIRUS (HBV) infects man at a very high rate. In western Europe and USA, about 10% of the population carry an HBV serological marker such as the surface antigen (HBs Ag) or the corresponding antibody (HBs Ac)[1]. In some areas of tropical Africa and southeastern Asia, this percentage can be as high as 60% or 70% (ref. 2). Recovery is spontaneous in most cases, but the virus can cause very severe complications like fulminant hepatitis, chronic hepatitis and cirrhosis in addition to which a hepatocarcinoma can develop[3]. It has been estimated that there are at least 120 million chronic carriers of HBs Ag, the so-called healthy carriers, throughout the world[2].

In addition to HBs Ag, two other antigens have been identified: HBc Ag which corresponds to the core of the virus, and HBe Ag whose location within the virion has been recently demonstrated[4]. It was also shown that the viral genome is made up of a circular DNA molecule which is partially single-stranded and about 3,200 bases long[5,6]. The virion also harbours a DNA polymerase able to repair the viral DNA[7]. The novel structure of this virus makes its classification somewhat difficult.

The biological study of the virus is complicated by the fact that HBV cannot be propagated in cell cultures and the only available viral source remains human serum. However, cloning of the HBV genome in *Escherichia coli* has allowed the purification of large amounts of genetically homogeneous HBV DNA, and the establishment of several restriction maps[8–10]. Hence we have recently[11] localised and determined the primary structure of the *S* gene which codes for polypeptide I and its glucosylated derivative polypeptide II, which are the major components of the HBs Ag.

In this article, we report the complete primary structure of the viral genome (subtype ayw) previously cloned in *E. coli.*

Primary structure of the cloned viral genome

To determine the nucleotide sequence of this DNA, the chemical degradation method of Maxam and Gilbert[12,13] and the dideoxynucleotide method of Sanger *et al.*[14] as modified for

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† To whom correspondence should be addressed.

§ Present address: Unité de Génie Génétique, Institut Pasteur, Paris, France.

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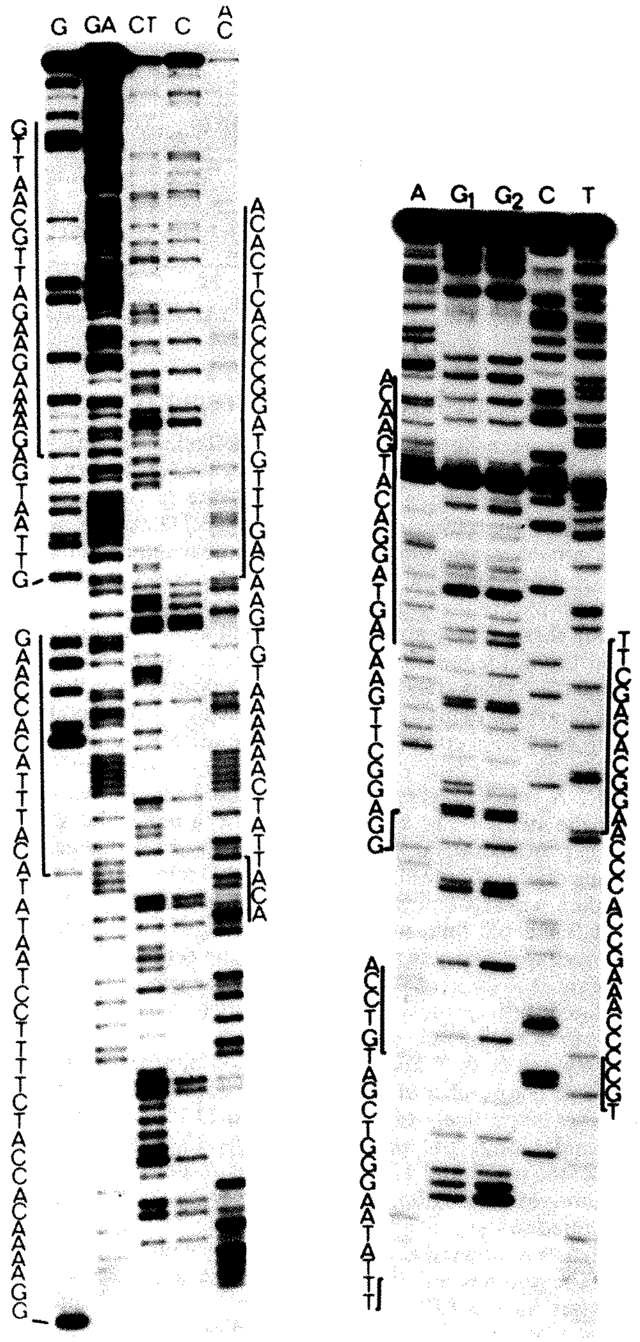
G
GA
CT
C
A C
GTTAACGTTAGAAGAAAAGAGTAATTG
GAACCACATTTACATATAATCCTTTCTACCACAAAGG
ACACTCACCCGGATGTTTGACAAGTGTAAAAAACTATTACA
A
G1
G2
C
T
ACAAGTACAGGATGACAAGTTCGGAGG
ACCTGTAGCTGGAATATT
TTCGACACGGAACCCACCGAAACCCCGT

Fig. 1 Viral DNA (serotype ayw), repaired with the endogenous DNA polymerase and the four nucleotide triphosphates, was digested with *Eco*RI endonuclease and cloned in *E. coli* using λ gt WES bacteriophage as the cloning vehicle[16]. The cloned DNA was referred to as *Eco* HBV DNA. To avoid genetic drift, all stocks used to analyse the DNA sequence were made on plates from the same lysate obtained after the first cloning step. Recombinant phages were purified by two successive CsCl gradients and the DNA was phenol extracted[11]. After hydrolysis with *Eco*RI endonuclease, *Eco* HBV DNA was separated from the two λ arms by sucrose gradient ultracentrifugation[17]. To prepare each set of restriction DNA fragments, 25 to 30 μg of *Eco* HBV DNA were fully hydrolysed with one of the restriction enzymes described in Fig. 2, using the conditions recommended by the manufacturer (Biolabs). Restriction fragments were dephosphorylated by incubation at 65 °C for 45 min with 4 μl of bacterial phosphatase. This enzyme was then inactivated by alkaline treatment[18] and DNA fragments were 5′ labelled with [γ-^{32}P]ATP (NEN; specific activity 2,500 Ci mmol^{-1}) and polynucleotide kinase (P. L. Biochemicals) as described by Maxam and Gilbert[12]. Labelled fragments were fractionated by gel electrophoresis and eluted from the gels as previously described. The two labelled ends of each restriction fragment were then separated either by hydrolysis of the DNA fragment with another restriction endonuclease and gel electrophoresis or by heat denaturation in the presence of 30% dimethyl sulphoxide and gel electrophoresis[13]. DNA fragments labelled at one 5′ end were eluted from the preparative gel and dissolved in water. Chemical reaction, specific for G, A + G, C + T, T and A + C giving a stronger band for A than C were performed as described previously[12,13,17]. Sequence reaction products dissolved in loading solution were heat denatured and fractionated on 25, 16 and 8% acrylamide gel. Figure 1*a* is an example of fractionation on an 8% acrylamide slab gel of the sequence reaction products obtained with *Hinf* C″ DNA fragment (nucleotides 658 to 552). When the two labelled ends were separated by hydrolysis with a restriction enzyme, nucleotide sequences were also determined with the dideoxynucleotide method[14] adapted by Maat and Smith[15] for double-stranded DNA fragments. Figure 1*b* is an example of fractionation on a 16% acrylamide gel of such reaction products obtained with *Bgl* A′ fragment (nucleotide sequence from residue 1,264 to residue 1,338). The two G lanes correspond to two different reaction conditions. In Gl there is no dGTP while in G2 the final concentration of dGTP is 2 μM. In both reactions, there is 1 mM ddGTP, final concentration. Subscript prime indicates the position of the ^{32}P on the L strand, subscript second, its position on the S strand.

double-stranded DNA by Maat and Smith[15] were used. Two examples of sequencing gel autoradiograms are shown in Fig. 1. To analyse independently both DNA strands and to overlap all the restriction sites used as starting points, a large number of fragments were analysed (Fig. 2). The localisation of the 5′ end of the largest DNA strand on the *Bam*HI viral restriction map and the knowledge of this restriction map on the *Eco* HBV DNA allowed the identification of the cloned DNA strands with respect to the viral DNA strands. Here we call the DNA strand of *Eco* HBV DNA homologous to the longest viral DNA strand the L strand, and the other, the S strand. The sequence shown in Fig. 3 corresponds to the *Eco* HBV DNA, which is 3,182 nucleotides long.

Location of the open reading frames on the viral genome

A computer program was used to search for nonsense codons on both DNA strands[19]. It appears from Fig. 4 that the number and distribution of nonsense codons are very different on S and L strands. Considering the total genome length, a random distribution of nucleotides should give about 50 nonsense codons in each reading frame. Instead one observes 33, 29 and 15 nonsense codons in the S strand for each phase and 61, 60 and 41 nonsense codons on the L strand. In addition, their distribution along the DNA molecule is very different on the two strands. On the L strand, the open reading frames are relatively short (Fig. 4). Four regions are able to code for 100 amino acids or more. These four regions labelled 1 to 4 are 510, 327, 447 and 345 nucleotides long. On the S strand (Fig. 4), the open regions are much longer. These regions labelled 5 to 8 are 498, 2,553, 1,296 and 657 nucleotides long. The length of the above hypothetical coding regions which include the *Eco*RI restriction site (such as regions 4, 6 and 7) is only valid if no DNA has been lost in the vicinity of this restriction site on cloning, which is most likely. In spite of this limitation, the distribution of stop codons clearly shows that the coding capacity of the L strand is larger than that of the S strand.

Regions 1, 2, 3 and 4 appear to be located on the genome as two pairs (Fig. 5)—region 3 is located immediately downstream of region 1 and region 2 immediately beyond region 4. Regions 5, 6, 7 and 8 extend over the whole genome and overlap with each other in different reading frames (Fig. 5). Region 6 covers about 80% of the genome and includes the whole of region 7 and part of regions 5 and 8. Regions 6 and 7 correspond partly to the single-stranded region of the genome. Regions 4 and 5 cover the 5′ extremity of the S strand and the nick of the L strand.

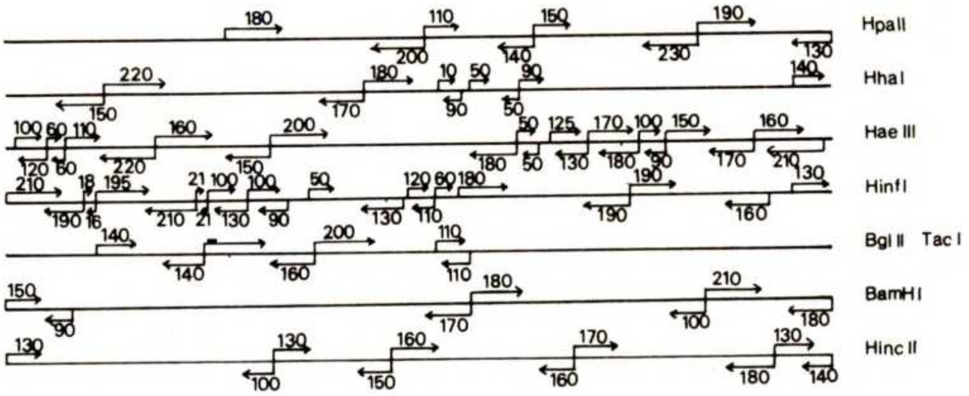

Fig. 2 Diagram of analysed DNA fragments. Vertical bars correspond to the position of the 5′ labelled ends of restriction fragments used to determine the nucleotide sequence of the *Eco* HBV DNA. Numbers above each arrow indicate the length of nucleotide sequences analysed from the restriction sites.

Tentative translation of open regions

Few proteins have been shown to be coded by the viral genome[9,11], but one can suppose that, because of its very small size, regions with open reading frames are largely used for translation. Therefore, a search for the ATG codons which could initiate translation has been made near the beginning of these regions, although the existence of mRNA splicing[20,21] could complicate the translation of the open regions. The positions of these ATGs are shown by small arrows in Fig. 5. In region 1 to 4, the numbers of codons located downstream from these ATG codons are 79, 60, 145 and 28 respectively. Regions 2 and 4 have particularly low coding capacities. However, these coding capacities could be modified by mRNA splicing. For example, the translation from the first ATG located in region 4, of mRNA transcribed from regions 4 and 2 and spliced could allow the synthesis of a polypeptide with a molecular weight (MW) up to 18,000. For simplicity and clarity, only regions with an open reading frame able to code for 100 amino acids or more are taken into account. But a more complicated splicing pattern of mRNA could link, in one open reading frame, areas with small coding capacity scattered all along the genome. If region 3 which spans in the single-stranded part of the genome is actually translated into protein, this can happen only after DNA synthesis of the missing strand.

In regions 5, 6, 7 and 8, the situation appears to be very different. The number of codons located downstream of the first ATG are respectively 154, 832, 389 and 212. Region 6 (2,553 nucleotides long) contains 9 ATG codons. The first and second are located at 57 and 1,296 nucleotides respectively from the beginning of this region. The seven other ATGs are regularly distributed in the second half of this region. In the case where translation is initiated at the first ATG, a protein of MW 90,000 might be synthesised. DNA polymerases and reverse transcriptases have a MW of that order[22,33], so it is tempting to suggest that the DNA polymerase found within the virion could be coded by this region.

By correlating the amino acid sequences of both ends of the HBs Ag polypeptide I[24] and the nucleotide sequence of the *Eco* HBV DNA, we are able to localise the gene coding for this polypeptide in region 7. This gene, called *S* gene, was previously located between positions, 95.1 and 73.6 on the genome map[11]. The ATG (position 3,030) corresponding to the N-terminal extremity of the polypeptide I is the third of region 7. It is located 618 nucleotides from the beginning of this region, whereas the two other ATGs (positions 337 and 13) are at 129 and 453 nucleotides from the beginning. The fact that the ATG 3030 is preceded by two other ATGs in the same open reading frame deserves some additional comment. Three explanations can be given: (1) Region 7 is completely translated and gives rise to a long polypeptide different from polypeptide I but whose C-terminal amino acid sequence is identical to polypeptide I. A similar situation occurs with protein VP2 and VP3 of SV40[25,26] and the 33,000 and 100,000 MW proteins of adenovirus 2[27]. (2) Region 7 is completely translated into a precursor protein of polypeptide I. (3) The region upstream of the third ATG is not translated. At the present time, one cannot choose between the

Fig. 3 (Below and opposite) Nucleotide sequence of *Eco* HBV DNA. Stop codons in the various reading frames are indicated in the following manner: ○ stop codon in phase 1; ● in phase 2; + in phase 3. The position of *Hin*cII, *Bam*HI, *Bgl*II and *Xho*I restriction sites are indicated.

```
5                +20 +          40          ○ 60         ●          80
AATTCCACTGCATGGCCTGAGGATGAGTGTTTCTCAAAGGTGGAGACAGCGGGGTAGGCTGCCTTCCTGACTGGCGATTG
TTAAGGTGACGTACCGGACTCCTACTCACAAAGAGTTTCCACCTCTGTCGCCCCATCCGACGGAAGGACTGACCGCTAAC
                                   +
3

               100           ● 120    +    +     140                       160
GTGGAGGCAGGAGGCGGATTTGCTGGCAAAGTTTGTAGTATGCCCTGAGCCTGAGGGCTCCACCCCAAAAGGCCTCCGTG
CACCTCCGTCCTCCGCCTAAACGACCGTTTCAAACATCATACGGGACTCGGACTCCCGAGGTGGGGTTTTCCGGAGGCAC

        ○      180             200              220                    ○ 240
CGGTGGGGTGAAACCCAGCCCGAATGCTCCAGCTCCTACCTTGTTGGCGTCTGGCCAGGTGTCCTTGTTGGGATTGAAGT
GCCACCCCACTTTGGGTCGGGCTTACGAGGTCGAGGATGGAACAACCGCAGACCGGTCCACAGGAACAACCCTAACTTCA
                                    +

               260      ○      280              300                        320
CCCAATCTGGATTTGCGGTGTTTGCTCTGAAGGCTGGATCCAACTGGTGGTCGGGAAAGAATCCCAGAGGATTGCTGGTG
GGGTTAGACCTAAACGCCACAAACGAGACTTCCGACCTAGGTTGACCACCAGCCCTTTCTTAGGGTCTCCTAACGACCAC
                                   BamHI

               340 +           360     +         +380                      400
GAAAGATTCTGCCCCATGCTGTAGATCTTGTTCCCAAGAATATGGTGACCCACAAAATGAGGCGCTATGTGTTGTTTCTC
CTTTCTAAGACGGGGTACGACATCTAGAACAAGGGTTCTTATACCACTGGGTGTTTTACTCCGCGATACACAACAAAGAG
                     BglII                                       +

               420    +      + ○440    ●        ○460○  ●  +                    480
TCTTATATAATATACCCGCCTTCCATAGAGTGTGTAAATAGTGTCTAGTTTGGAAGTAATGATTAACTAGATGTTCTGGA
AGAATATATTATATGGGCGGAAGGTATCTCACACATTTATCACAGATCAAACCTTCATTACTAATTGATCTACAAGACCT
   +                                   +              +   ●

○  ○   ●       500        +       520●       ○      540      ●                 560
TAATAAGGTTTAATACCCTTATCCAATGGTAAATATTTGGTAACCTTTGGATAAAACCTGGCAGGCATAATCAATTGCAA
ATTATTCCAAATTATGGGAATAGGTTACCATTTATAAACCATTGGAAACCTATTTTGGACCGTCCGTATTAGTTAACGTT
            +       +                                            +   ●

              ● 580●            600          ●  ●   620      +                ●640
TCTTCTTTTCTCATTAACTGTGAGTGGGCCTACAAACTGTTCACATTTTTTGATAATGTCTTGGTGTAAATGTATATTAG
AGAAGAAAAGAGTAATTGACACTCACCCGGATGTTTGACAAGTGTAAAAAACTATTACAGAACCACATTTACATATAATC
            +  +            ●                       +                          +

               660○      ●      680    ●      ○      700●                    ○720
GAAAAGATGGTGTTTTCCAATGAGGATTAAAGACAGGTACAGTAGAAGAATAAAGCCCAGTAAAGTTCCCCACCTTATGA
CTTTTCTACCACAAAAGGTTACTCCTAATTTCTGTCCATGTCATCTTCTTATTTCGGGTCATTTCAAGGGGTGGAATACT
                          +                                                   +

             ●  740●        ●  760                     780●                    800
GTCCAAGGAATACTAACATTGAGATTCCCGAGATTGAGATCTTCTGCGACGCGGCGATTGAGACCTTCGTCTGCGAGGCG
CAGGTTCCTTATGATTGTAACTCTAAGGGCTCTAACTCTAGAAGACGCTGCGCCGCTAACTCTGGAAGCAGACGCTCCGC
              +                          BglII

              + 820              +840     +            860 ○  ○   +            880
AGGGAGTTCTTCTTCTAGGGGACCTGCCTCGTCGTCTAACAACAGTAGTCTCCGGAAGTGTTGATAGGATAGGGGCATTT
TCCCTCAAGAAGAAGATCCCCTGGACGGAGCAGCAGATTGTTGTCATCAGAGGCCTTCACAACTATCCTATCCCCGTAAA
                ●                   ●

         ●     900              920              +940                         +960
GGTGGTCTATAAGCTGGAGGAGTGCGAATCCACACTCCGAAAGACACCAAATACTCTATAACTGTTTCTCTTCCAAAAGT
CCACCAGATATTCGACCTCCTCACGCTTAGGTGTGAGGCTTTCTGTGGTTTATGAGATATTGACAAAGAGAAGGTTTTCA
        ●                                                ○

             ●   980         +  1000○         +  +1020 ●   +    ○             1040
GAGACAAGAAATGTGAAACCACAAGAGTTGCCTGAACTTTAGGCCCATATTAGTGTTGACATAACTGACTACTAGGTCTC
CTCTGTTCTTTACACTTTGGTGTTCTCAACGGACTTGAAATCCGGGTATAATCACAACTGTATTGACTGATGATCCAGAG
                                         ○         ●    HincII        ○  ○

+              1060 +           +1080○         ○     1100                     1120+
TAGACGCTGGATCTTCCAAATTAACACCCACCCAGGTAGCTAGAGTCATTAGTTCCCCCCAGCAAAGAATTGCTTGCCTG
ATCTGCGACCTAGAAGGTTTAATTGTGGGTGGGTCCATCGATCTCAGTAATCAAGGGGGGTCGTTTCTTAACGAACGGAC
 ●                   ●                     ○    ○  ○

            ●  1140○          1160○             1180○               ○         1200
AGTGCAGTATGGTGAGGTGAACAATGCTCAGGAGACTCTAAGGCTTCCCGATACAGAGCTGAGGCGGTATCTAGAAGATC
TCACGTCATACCACTCCACTTGTTACGAGTCCTCTGAGATTCCGAAGGGCTATGTCTCGACTCCGCCATAGATCTTCTAG
                             ●         ○                                ○
                                                                      BglII

      ○        1220             1240○       +     1260         ●              1280
TCGTACTGAAGGAAAGAAGTCAGAAGGCAAAAACGAGAGTAACTCCACAGTAGCTCCAAATTCTTTATAAGGGTCGATGT
AGCATGACTTCCTTTCTTCAGTCTTCCGTTTTTGCTCTCATTGAGGTGTCATCGAGGTTTAAGAAATATTCCCAGCTACA
                     ●                                             ●
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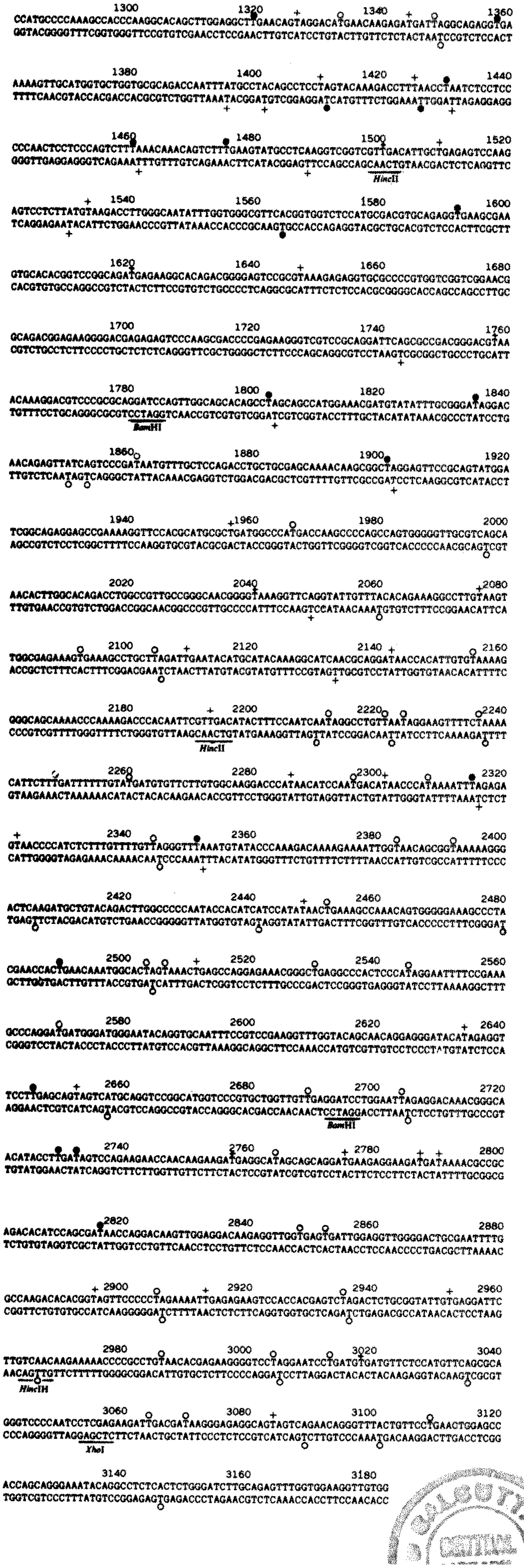

three hypotheses. Nevertheless, the first hypothesis could partially explain the presence of large polypeptides found in small amount in the HBs Ag preparations[24].

Translation of the terminal nucleotide sequence of region 8 would give rise to a polypeptide having at its C-terminal end a repeated sequence of 9 amino acids: Ser-Pro-Arg-Arg-Arg-Arg-Ser-Glu-Ser. The last amino acid of the first sequence, Ser, would be the first one of the second sequence. In the corresponding DNA sequence, however, the only repetition is TCTCAA (Ser-Glu). Beyond the ATG codon, region 8 contains 24 arginine codons—11% of the total codons. In the last 35 codons there are 16 arginine codons. This large proportion of basic amino acids is similar to that observed in histone, which suggests that the translational product of region 8 could be a polypeptide with a high affinity for HBV DNA. Therefore, if this region is translated, one could envisage that the HBc Ag could be coded by this DNA sequence.

Search for signal sequences

From a comparative analysis of the primary structure of viral or cellular mRNAs and of chromosomal DNA fragments, some remarkable DNA sequences were found. In several circumstances, a sequence identical or similar to 5'TATAAA was observed in front of the transcribed region (ref. 28 and D. Hogness and M. Goldberg, personal communication), while the sequence AAUAAA has been found at the 3' end of mRNA, near the poly(A)tail[29–31]. On the other hand, it has been shown that during the initiation of translation the 3'OH end of 16S ribosomal RNA hybridises to the 5' end of prokaryotic mRNA through the CCUCC sequence[32]. The occurrence of a similar phenomenon has not been demonstrated in eukaryotic cells. Nevertheless, sequences able to hybridise to the 3'GCGGAAGGA sequence of the 18S ribosomal RNA have been observed in some eukaryotic mRNAs[28,33–35]. Therefore, we looked for these sequences in the *Eco* HBV DNA. The 5'TATAAA sequence was found only at position 1,269 on the S strand at 335 nucleotides before region 6. It could therefore correspond to a signal relating to the initiation of transcription of this very large open region. In that case, since this sequence is followed by three ATGs (1,089, 1,022, 998) all in a closed reading frame, a splicing of the corresponding mRNA should

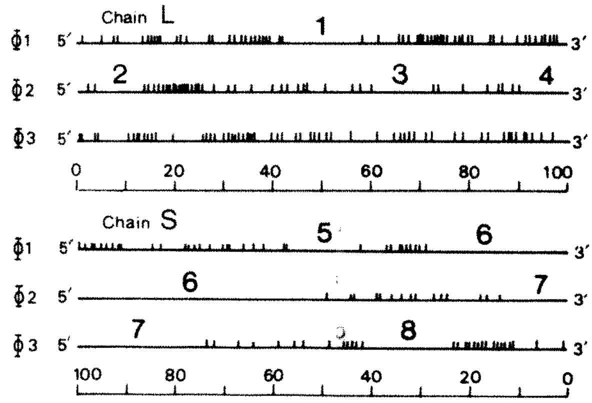

Fig. 4 Diagram showing the localisation of the nonsense codons on chains L and S of the *Eco*HBV DNA. Three reading frames, called Φ1, Φ2 and Φ3, were defined from the 5' end of each DNA strand. On the L chain, Φ1 is defined by its first triplet AAT, Φ2 by ATT and Φ3 by TTC. On the S chain, Φ1 is defined by its first triplet CCA, Φ2 by CAC and Φ3 by ACA. The viral DNA is circular and its length in nucleotides (3,182) is not a multiple of 3. Therefore, going through the *Eco*RI site phase Φ1 is change to phase Φ2, phase Φ2 to phase Φ3 and phase Φ3 to phase Φ1. Vertical bars represent stop codons. Numbers 1 to 8 define the areas with an open reading frame arbitrarily chosen with a length above 100 codons. Region 1 goes from nucleotide 1,351 to 1,860, region 2 from nucleotide 119 to 445, region 3 from 1,904 to 2,350, region 4 from 2,855 to 17, region 5 from 1,847 to 1,350, region 6 from 935 to 1 and from 3182 to 1565, region 7 from 466 to 1 and from 3,182 to 2353, region 8 from 1,392 to 736.

CALCUTTA

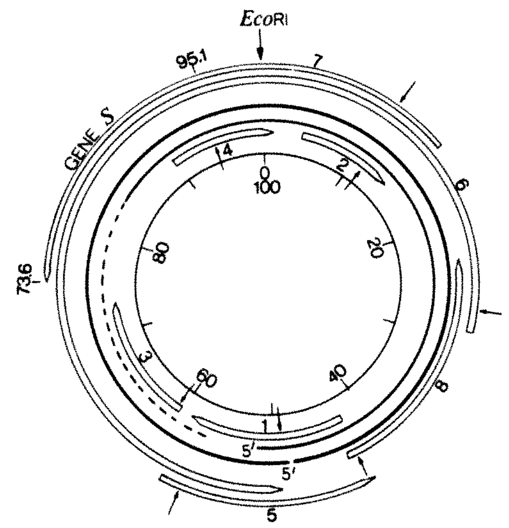

Fig. 5 Localisation of the open reading frames on the viral genome. The structure of the genome is shown as previously described[8]. The *Eco*RI site is the origin of the physical map. Block arrows define the open reading frames on the two viral DNA strands. The small arrows indicate the ATG codon located closest to the beginning of the open region. The *S* gene is located between positions 95.1 and 73.6 and is included in region 7.

take place to link its 5′ end to a nucleotide sequence corresponding to the open reading frame of region 6. Sequences which differ by only one nucleotide were found 36 times on the S strand and 45 times on the L strand. The 5′AATAAA sequence occurs once on the L strand at position 689, 243 nucleotides after the stop codon of region 2. This sequence is not found in chain S even at the end of gene *S* which is known to code for polypeptide I. Two sequences, 5′GCCTTCCT and CGCCTTCC, able to hybridise with the 3′ end of 18S RNA ribosomal, were found at positions 61 and 417 on the L strand. Sequences able to hybridise with 6 or 7 nucleotides of the 3′OH end of this RNA were found once on the L chain and 8 times on the S chain. Among these, the CCTTCC sequence located at position 3,176 on the S strand follows a short palindromic sequence 5′GTGGAATTCCAC 3′ starting at position 8. This feature could be related to the expression of the *S* gene located downstream.

Containment conditions for this study were recommended by the French National Control Committee. Growth of recombinant bacteriophages was done in L3B1 conditions. We thank Dr A. Fritsch for helpful discussion and reviewing the manuscript, Dr R. Studen for computer work, Dr A. Maxam for sending us the revised version of the Maxam and Gilbert sequencing method before publication, and Mrs Gobet for technical assistance. This work was supported by INSERM and CNRS.

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Design, synthesis and characterisation of a 34-residue polypeptide that interacts with nucleic acids

B. Gutte, M. Däumigen & E. Wittschieber
Max-Planck-Institut für Biophysikalische Chemie, Abteilung Biochemische Kinetik, 34 Göttingen, FRG

Based on secondary structure prediction rules and model building a neutral artificial 34-residue polypeptide with potential nucleic acid-binding activity was synthesised. This peptide and its covalent dimer showed strong interaction with cytidine phosphates and single-stranded DNA. The dimer had considerable ribonuclease activity with high preference for cleavage at the 3′-end of C.

BASED on the known X-ray structures of proteins, rules were proposed by various authors (see ref. 1 for review) to describe the relationship between primary and secondary structure of a protein molecule. These rules may be applied to predict the secondary structure of proteins of which only the amino acid sequence is known. An example of this is the suggested succession of the secondary structural elements (α-helix, β-strand, reverse turn) in *Escherichia coli lac* repressor[2,3]. Proof or disproof of the proposed secondary structure will come from X-ray diffraction studies on crystalline *lac* repressor.

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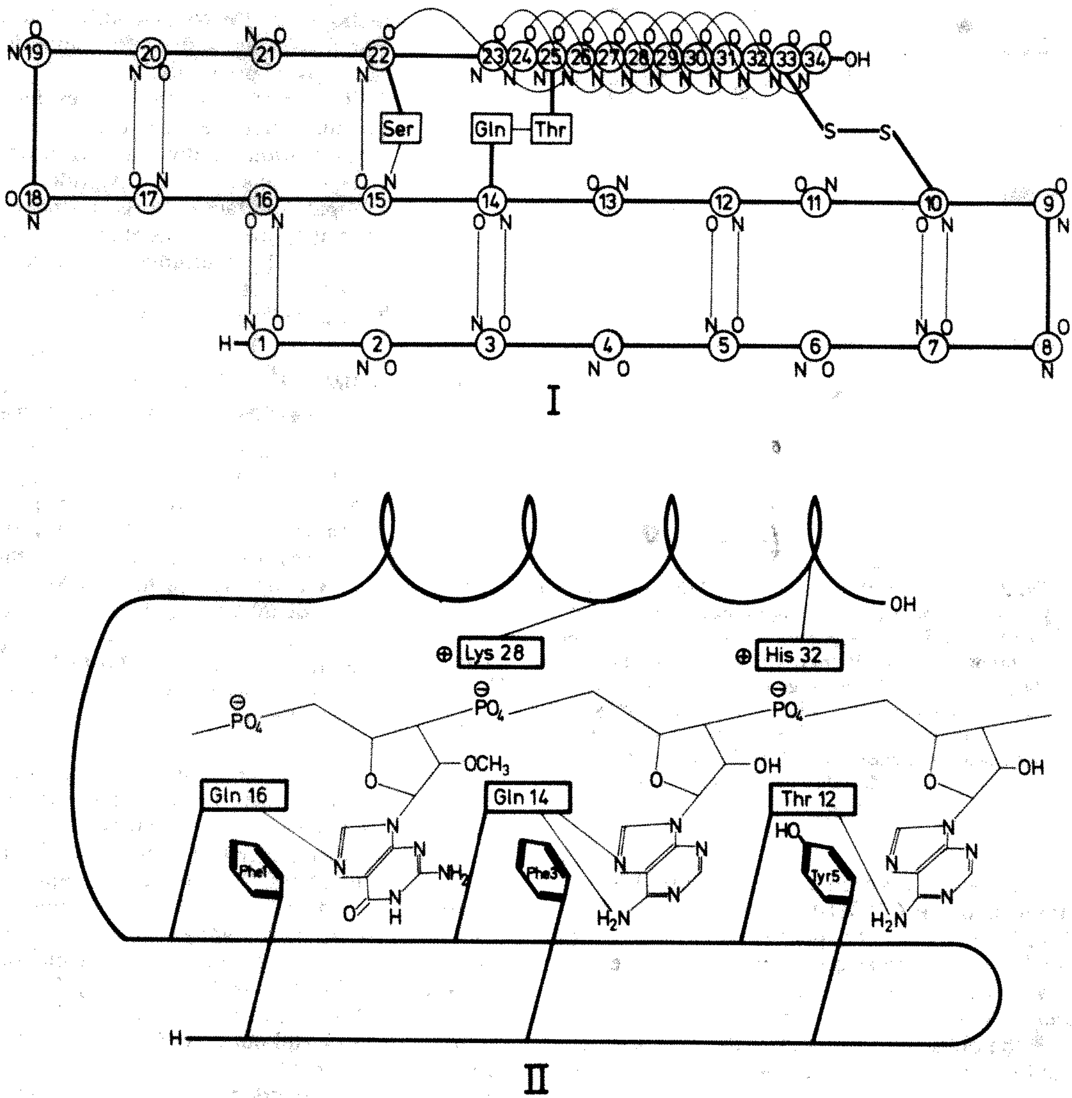

Fig. 1 Proposed secondary structure of synthetic 34-residue polypeptide (I) and possible interactions with the trinucleotide m$^{2'}$ GAA (II). I, Thin lines represent hydrogen bonds between the β-strands and within the α-helix, amino acid symbols in boxes are side chains involved in intramolecular hydrogen bonds. In the model, the helix axis lies slightly above the hydrophobic side of the β-sheet. The primary structure of the 34-residue polypeptide is given in the text. II, Heavy lines indicate the course of the peptide chain, thin lines show the trinucleotide. The side chains of the amino acid residues in boxes are proposed to be the main contributors to the interactions between the synthetic polypeptide and the trinucleotide. The ribose–phosphate backbone runs above the two parallels formed by the 10–17 strand and the helix axis. The bases point downwards onto the two-strand sheet formed by residues 1–7 and 10–17.

The secondary structure prediction rules may also be used to design functional polypeptides that do not occur naturally but that may be useful for scientific and industrial purposes. Although these rules will certainly become refined and more reliable as more X-ray structures of proteins become known, we decided to design a nucleic acid-binding polypeptide based on the present prediction methods. This system was chosen because of the great importance of nucleic acid–protein interactions in biology[4].

Rationale of the amino acid sequence chosen

The structure and the amino acid sequence of the polypeptide that could have the desired properties were determined in part by model building. The nucleotide sequence of the anticodon of yeast tRNA^{Phe}, m$^{2'}$ GAA (refs 5–7), was used as the ligand around which the backbone of a peptide chain was to be folded in such a way as to produce a shallow depression as binding site. This simple structure seemed to be formed best by a 34-residue polypeptide containing a β-strand, a reverse turn, an antiparallel β-strand, a second reverse turn, and an α-helix running antiparallel to the second β-strand as viewed from the NH_2-terminus to the COOH-terminus. Although this $\beta\beta\alpha$ arrangement was similar to the $\beta\alpha\beta$ units that make up the NAD$^+$-binding fold of the dehydrogenases[8,9], the proposed mode of interaction between the synthetic polypeptide and the trinucleotide was quite different.

The next step was choosing an amino acid sequence that, based on the prediction method of Chou and Fasman[10], had the potential to give the proposed folding and to form a sufficient number of specific noncovalent bonds with the ligand. Two additional prerequisites had to be fulfilled. First, the binding site was to be predominantly hydrophobic containing a few residues the side chains of which could act as hydrogen bond donors or acceptors; and second, binding was to be achieved mainly through specific hydrogen bonds and van der Waals contacts and only to a smaller extent through electrostatic interactions. The sequence chosen was as follows:

1 5 10
Phe-Thr-Phe-Thr-Tyr-Thr-Asp-Pro-Asn-Cys-Gln-Thr-Gly-
15 20 25
Gln-Gly-Gln-Asn-Pro-Asn-Gly-Ile-Ser-Glu-Pro-Thr-Ala-
30 34
Ala-Lys-Val-Ala-Gln-Ala-His-Cys-Ala

The proposed secondary structure of this peptide and its possible interactions with the ligand are shown in Fig. 1. Residues Phe 1 to Asp 7 were assumed to have an extended structure with the side chains of Phe 1, Phe 3, and Tyr 5 forming part of the binding site and allowing stacking interactions or intercalation with the bases of the trinucleotide ligand and the side chains of Thr 2, Thr 4, and Thr 6 pointing in the opposite direction, thus allowing interactions with the solvent. Residues 7–10 could form a β-turn and residues 10 to 17 could form the second strand of a β-sheet with the side chains of Thr 12, Gln 14, and Gln 16 hydrogen-bonded to the base moieties of the trinucleotide in a hydrophobic environment. Following the second

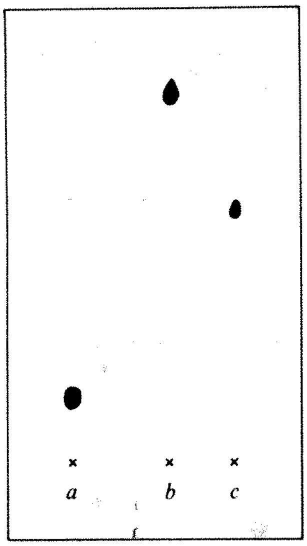

Fig. 2 Electrophoresis (*a*) and TLC (*b*, *c*) of purified synthetic 34-residue polypeptide. Electrophoresis was in 0.1 M pyridine-acetate, *p*H 5.8, on a precoated cellulose thin layer plate (20 × 20 cm) at 600 V for 50 min. The mobility of the spot was 2.3 cm. The product was also homogeneous on electrophoresis at *p*H 3.4. TLC was on precoated silica gel plates in ethyl acetate/acetic acid/pyridine/water (3:1:5:3) (*b*, R_F 0.80) and in *n*-butanol/acetic acid/pyridine/water (10:3:10:12) (*c*, R_F 0.50). Spots were detected by the Pauly spray.

β-turn, possibly produced by residues 17–20, a short third strand and the COOH-terminal α-helix comprising residues 23–34 completed the proposed secondary structure of the synthetic 34-residue polypeptide. The side chains of Ile 21, Ala 26, and Val 29 could be part of the hydrophobic binding site and the side chains of Lys 28 and His 32 could form salt bridges with the phosphate moieties of the ligand although the contribution of the latter interactions to the stability of the peptide–trinucleotide complex was probably weak, as in the model these salt bridges were on the surface of the complex. The disulphide bond between half-cystines 10 and 33 seemed to stabilise the proposed structure of the 34-residue polypeptide and its interactions with the trinucleotide. It also brought the side chains of three potential active site residues (Asp 7, Thr 12, and His 32) into spatial proximity.

The model of the 34-residue polypeptide–trinucleotide complex suggested the possibility of aggregation, either through continuation of the stacking interactions to the left of the NH_2-terminus of the peptide (Fig. 1, II) or through an antiparallel face-to-face arrangement of the NH_2-terminal heptapeptide portions of two 34-residue polypeptide molecules mediated by intermolecular hydrogen bonds between residues 2, 4 and 6 of one molecule (Fig. 1, I) and residues 6, 4 and 2, respectively, of the other.

Solid phase synthesis and purification of the 34-residue polypeptide

The 34-residue polypeptide was synthesised by the solid phase method[11,12] as described previously[13]. The amino acid composition of an acid hydrolysate of the protected peptide resin is given in Table 1. The 34-residue peptide was deprotected and cleaved from the resin by anhydrous HF in the presence of excess anisole at 0 °C and was extracted by 1 M acetic acid. After lyophilisation it was dissolved in 0.05 M NH_4HCO_3 adjusted to *p*H 8.8 with concentrated NH_4OH. The solution was stirred several hours and then desalted on a Bio-Gel P-2 column. The yield of crude 34-residue peptide was 67% based on the amount of COOH-terminal alanine esterified to the resin at the beginning of the synthesis.

The synthetic product was reduced with mercaptoethanol, reoxidised in dilute solution at *p*H 8.55 (ref. 13), and fractionated on a Sephadex G-50 column (78.5 × 2.7 cm) in 0.05 M NH_4HCO_3. The major fractions obtained were a polymer (yield, 5% of the amount applied to the column), a tetramer (yield, 19%), and the monomer (yield, 41%) which, after rechromatography, eluted between 136 and 154 ml, 211 and 247 ml (corresponding to the elution volume of lysozyme), and 272 and 308 ml, respectively. When 1 M HCOOH was used as eluant, the elution volume of the tetramer was increased to 240 to 270 ml but that of the monomer remained unchanged. This indicated that the tetramer was formed through association of two dimers the monomeric subunits of which were linked covalently by two intermolecular disulphide bonds. Whether the S–S bridges extended between Cys 10 and Cys 33 of one subunit and Cys 10 and Cys 33 or Cys 33 and Cys 10, respectively, of the

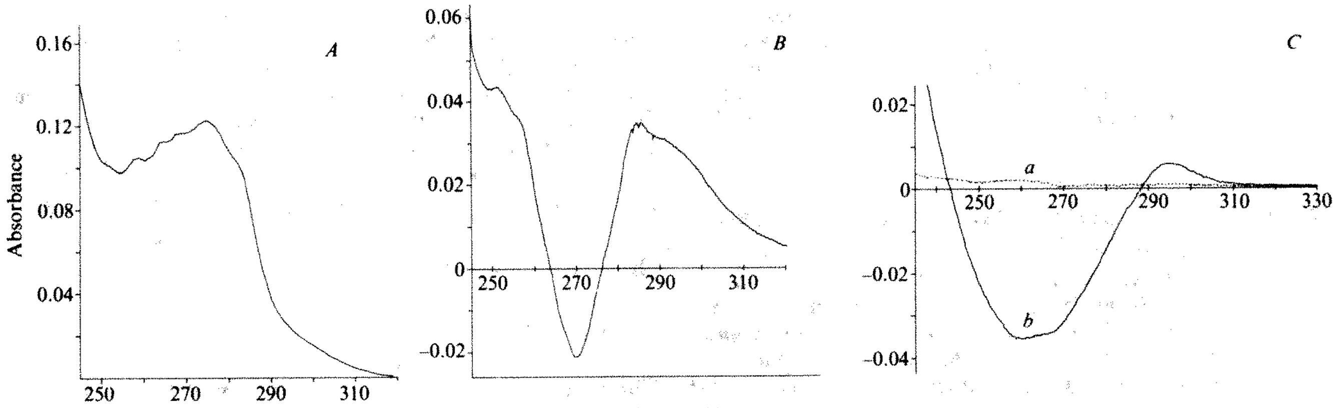

Fig. 3 *A*, *B*, UV difference spectrum of synthetic 68-residue polypeptide in the presence of cytidine 2′:3′-cyclic phosphate. Measurements were performed in a Cary 118 spectrophotometer. *A*, Spectrum of the 68-residue polypeptide (0.02 mM solution in 0.1 M sodium acetate, *p*H 5.3; *d*, 1 cm). *B*, Spectrum of the mixture after addition of the nucleotide to a concentration of 0.1 mM in both the control and the sample. *C*, UV difference spectrum of synthetic 34-residue polypeptide in the presence of single-stranded DNA. Single-stranded DNA was prepared from double-stranded calf thymus DNA by alkali denaturation. The 34-residue polypeptide was dissolved in buffer, *p*H 6.0, containing 0.06 M sodium chloride, 0.04 M sodium cacodylate, and 0.001 M EDTA. The separate compartments of two tandem cuvettes (*d*, 0.877 cm) were filled with 0.75 ml DNA solution (0.034 mg ml^{-1}) and 0.75 ml of peptide solution (0.24 mg ml^{-1}), respectively. After recording the base line (*a*), the contents of the two compartments of one of the cuvettes were mixed and the spectrum of the mixture was measured against that of the separate components (*b*). The UV difference spectrum produced by a mixture of the 68-residue material and single-stranded DNA was almost identical to that shown here.

other subunit or whether a mixture of both disulphide isomers was present, has not yet been determined.

Further purification of the 34-residue polypeptide and its dimer was achieved best by adsorption chromatography of 10-mg samples on a silica gel column (50×2.2 cm) in *n*-butanol/acetic acid/pyridine/water (20:6:20:24). The monomeric material that had the required amino acid composition (Table 1) was eluted between 102 and 109 ml in 35% yield based on the amount applied to the column. The major impurity eluting between 114 and 122 ml was a deletion peptide in which one threonine and one phenylalanine residue were missing. The purified monomer was homogeneous electrophoretically and chromatographically (Fig. 2). Fractionation of the dimer on silica gel gave similar results.

Interaction of the synthetic polypeptides with cytidine phosphates and single-stranded DNA

To visualise possible interactions between the purified synthetic 68- and 34-residue polypeptides and the $2':3'$-cyclic phosphates of adenosine, cytidine, guanosine, and uridine, UV spectra were recorded in a Cary 118 double-beam spectrophotometer. First, the 68-residue polypeptide was measured against the solvent, 0.1 M sodium acetate, *p*H 5.3. Then the nucleotide was added to both cuvettes in a small volume of buffer and the spectrum was recorded again between 320 and 250 nm. There was almost no change of the spectrum of the peptide in the presence of adenosine, guanosine, or uridine phosphate. In the presence of cytidine $2':3'$-cyclic phosphate, however, the spectral change was drastic (Fig. 3, *A*, *B*). The absorption maximum of the peptide was shifted from 274.5 to 285 nm and was quenched considerably. The spectrum of the mixture also showed a large negative peak approximately at the position of the absorption maximum of the nucleotide alone (268 nm). Addition of cytidine $2':3'$-cyclic phosphate to the 34-residue peptide and to its [Ala^{10}, Ala^{33}]-analogue which cannot form disulphide bonds, produced only a small decrease of the optical absorption of the two peptides.

The strong interaction between the 68-residue polypeptide and cytidine phosphate indicated by the UV difference spectrum (Fig. 3, *A*, *B*) was confirmed through determination of the dissociation constant of the polypeptide–nucleotide complex. Using the method of Hummel and Dreyer[14] and varying the concentration of 2′-CMP from 0.02 mM to 0.15 mM (in the presence of 0.1 mM 2′-CMP, for instance, the concentration of the polypeptide–ligand complex was 9.9×10^{-6} M and that of free polypeptide 5×10^{-7} M), the K_D was found to be 5×10^{-6} M in 0.01 M sodium acetate, *p*H 5.3 and was of the same order of magnitude as the K_D of the RNase A–2′-CMP complex[15]. By this method the 68-residue peptide bound 2′-CMP 30 times more strongly than did the 34-residue peptide.

The binding properties of the synthetic peptides were investigated further by affinity chromatography. Poly(A)–Sepharose 4B did not retard the elution of the 34-residue polypeptide but retarded considerably part of the 68-residue polypeptide applied—75% of the dimer was eluted at *p*H 5.3 at a concentration of 0.03 M to 0.07 M sodium acetate, the remaining 25% was eluted at 0.1 M to 0.24 M sodium acetate, *p*H 5.3. No differences in amino acid composition could be detected, so the two fractions may represent conformational isomers, possibly the disulphide isomers, of the synthetic 68-residue polypeptide. Poly(U)–Sepharose 4B and Blue Sepharose CL-6B, which has some affinity for proteins[16] possessing the NAD^+-binding fold[8,9], did not bind either of the two synthetic peptides.

Difference spectral measurements showed that the absorption of the mixtures of 68- and 34-residue polypeptide with double-stranded calf thymus DNA deviated only slightly from the sum of the absorptions of the separate components. The UV difference spectra obtained in the presence of single-stranded DNA, however, indicated strong polypeptide–nucleic acid interactions (Fig. 3, *C*). The main features of these spectra were a new peak with the absorption maximum at 295 nm and a depression with the absorption minimum extending from 258 to 267 nm.

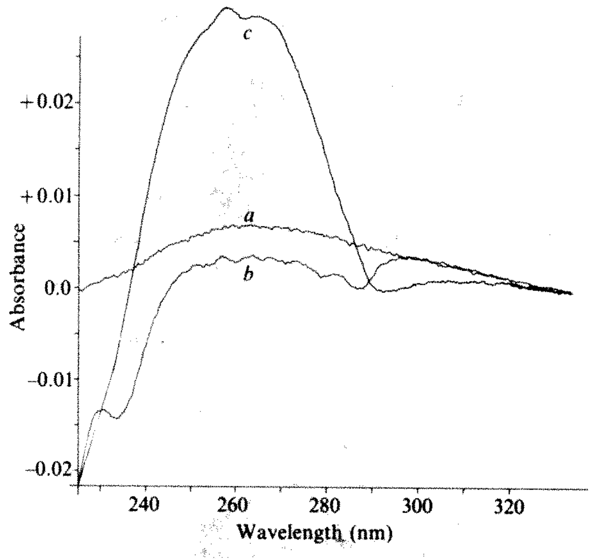

Fig. 4 Spectral evidence for the digestion of RNA by the synthetic 68-residue polypeptide. tRNA^{Phe} and 68-residue peptide were dissolved in buffer, *p*H 6.0, containing 0.1 M NaClO_4, 0.005 M sodium cacodylate, and 0.0001 M EDTA. The separate compartments of two tandem cuvettes (*d*, 0.877 cm) were filled with 0.75 ml of tRNA^{Phe} solution (0.35 μg ml^{-1}) and 0.75 ml of peptide solution (0.04 mg ml^{-1}), respectively. After recording the base line (*a*), the contents of the two compartments of one of the cuvettes were combined and the spectrum of the mixture was measured against that of the separate components immediately after mixing (*b*) and again 8 h later (*c*). The reaction took place at 22 °C.

Nuclease activity of synthetic 68- and 34-residue polypeptides

To test whether the synthetic peptides interacted with the ligand around which the model had been built, UV difference spectra of the mixtures of 68- and 34-residue polypeptide with tRNA^{Phe} in which the anticodon sequence m^2 GAA is in an exposed position were recorded.

Figure 4 shows that the spectral change observed immediately after combining solutions of 68-residue polypeptide and tRNA^{Phe} was small (curve *b*) indicating weak interaction between the two components of the mixture. However, when the spectrum was recorded again 8 h later a new broad peak with the maximum absorption at approximately 260 nm (curve *c*) was found. This type of spectral change is seen when RNA is digested by ribonucleases.

That the increase of the optical absorption around 260 nm was indeed due to a ribonuclease activity of the 68-residue polypeptide was confirmed by TLC of an aliquot of the contents of the cuvette. The digestion pattern obtained was similar to that shown in Fig. 5. In the Anfinsen assay[17,18] using tRNA from baker's yeast as substrate the specific activity of the synthetic 68-residue polypeptide was 2.5% that of natural RNase A.

Comparison of the patterns produced on digestion of tRNA by RNase A and the synthetic enzyme (Fig. 5, left and right, respectively) showed differences mainly between the products of high R_F or short chain length. This indicated that either the rates of the cleavage reactions or the substrate specificities of the two nucleases were different. Using poly(A), poly(C), poly(G) and poly(U) as substrates, differences in the cleavage rates and in the substrate specificity were indeed noticed in a semiquantitative assay. The 68-residue polypeptide digested the four polynucleotides with decreasing rates in the following order,

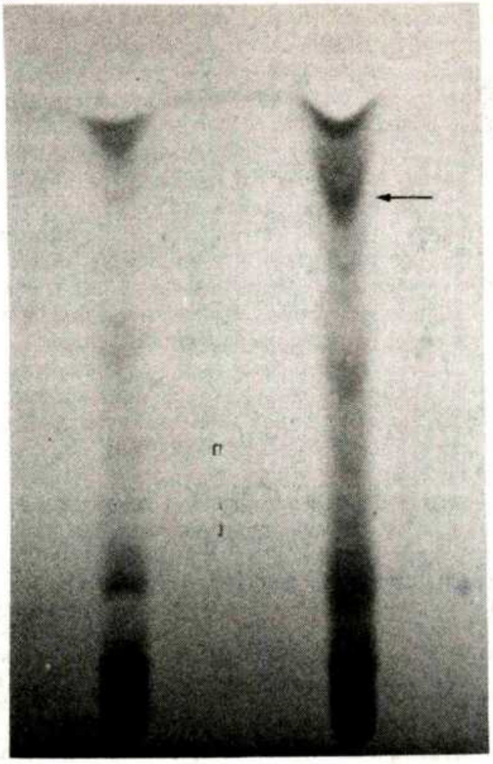

Fig. 5 TLC separation of the digestion products of tRNA produced by the action of synthetic 68-residue polypeptide (right) and natural RNase A (left). tRNA (0.20 mg each) was incubated with RNase A (0.1 μg) and 68-residue polypeptide (0.03 mg), respectively, in 30 μl of buffer, pH 6.0, at 22 °C. Aliquots (3 μl) of the reaction mixtures were chromatographed every 12 h until the digestion patterns remained unchanged (the patterns shown were obtained after a 93.5-h reaction time). Chromatograms were developed on silica gel F_{254}-coated thin layer plates in isopropyl alcohol/ammonium hydroxide/water (14:1:5). Spots were detected under UV light. The arrow indicates the position of free cytidine 2′:3′-cyclic phosphate. A control of undigested tRNA (not shown) gave a single spot at the origin. The buffer for the incubations contained 0.1 M $NaClO_4$, 0.005 M sodium cacodylate, and 0.0001 M EDTA, adjusted to pH 6.0.

poly(C) ≫ poly(A) > poly(U) ≫ poly(G). Using RNase A as enzyme, this order became poly(C) > poly(U) ≫ poly(A); poly (G) was not cleaved at all. The fact that cytidine phosphates were bound tighter by the 68-residue polypeptide than other nucleotides was reflected by the relatively fast depolymerisation of poly(C) in the presence of the synthetic enzyme. The nucleoside 2′:3′-cyclic phosphates seemed to be the final digestion products. The maximum of the rate of depolymerisation was between pH 6 and pH 8. But even in 0.1 M formic acid the 68-residue polypeptide had considerable nuclease activity; in identical conditions 0.1 M formic acid alone did not hydrolyse poly(C).

The activity of the two fractions obtained by affinity chromatography of the 68-residue polypeptide on poly(A)–Sepharose 4B was the same within the limit of error of the assay method using tRNA as substrate. Whether the tighter-binding fraction that eluted at sodium acetate concentrations of 0.1 to 0.24 M had increased nuclease activity towards poly(A) has not been tested.

The 34-residue polypeptide possessing one intramolecular disulphide bond was less than 4% as active as the 68-residue polypeptide. This was probably because of weaker binding of nucleic acids by the monomer. As controls, insulin and bovine serum albumin were completely inactive in these assays.

In assays for proteolytic (tryptic, chymotryptic, peptic) and glycolytic (lysozyme) activity both the 68- and the 34-residue polypeptide were inactive.

The possibility that the low ribonuclease activity of the 34-residue polypeptide might be due to incomplete separation from the more active 68-residue peptide was excluded by preliminary results obtained with an [Ala^{10}, Ala^{33}]-analogue of the 34-residue polypeptide. The nuclease activity of this analogue suggested that the disulphide bond was not essential for the structure and biological function of the 34-residue polypeptide and that the amino acid sequence chosen from model building could indeed fold in such a way as to allow interactions with nucleotides and nucleic acids. Dimerisation of the Cys-containing monomer through formation of two intermolecular disulphide bonds, however, seemed to produce a cleft or pocket in which the essential groups were in a more favourable position for binding and catalysis.

Chemical modification has been used successfully to determine active site residues and to propose catalytic pathways of ribonucleases[19]. Thus, iodoacetic acid was found to carboxymethylate at pH 5.5 the side chains of His 119 and His 12 of RNase A[20] and of Glu 58 of RNase T_1[21] to give inactive enzymes. When the synthetic 68-residue polypeptide was submitted to similar conditions[20] for 12 h, no carboxymethylation of the positively charged amino acid residues (Lys 28, His 32) took place as determined by electrophoresis and by amino acid analysis of an acid hydrolysate. The electrophoretic mobility of the 68-residue polypeptide, incubated with iodoacetic acid, on cellulose-coated thin layer plates in 1.2 M formic acid, 2 M urea, pH 2.2, was unaltered and no N^1- or N^3-carboxymethylhistidine was detected by amino acid analysis. Carboxymethylation of the 68-residue polypeptide at pH 8 gave identical results. However, alkylation of the side chain of Asp 7 or Glu 23 would have gone undetected by these methods. Therefore, the reaction was repeated using iodo[2-^{14}C]acetic acid. After 17 h at pH 5.75 only 1 out of 50 protein molecules seemed to be labelled. This was consistent with the result of the nuclease assay in which there was no noticeable loss of activity of the 68-residue polypeptide after iodoacetic acid treatment. Thus the geometry of the active site of the synthetic enzyme is apparently different from that of the active sites of RNase A and RNase T_1.

CD spectra of the synthetic polypeptides

To establish whether the synthetic peptides adopted the hypothetical secondary structure in aqueous solution spectral analyses were performed. The predicted structure (Fig. 1, I) suggested pronounced Cotton effects between 185 and 230 nm. In addition, the presence of phenylalanine and tyrosine residues in the NH_2-terminal region of the peptides could give rise to

Table 1 Amino acid composition of synthetic 34-residue polypeptide

	Expected	Protected peptide resin	Purified peptide
Asp	4	4.5	4.2
Thr	5	3.5*	5.1
Ser	1	0.5*	1.0
Glu	5	5.0	4.9
Pro	3	2.8	3.1
Gly	3	3.0	3.0
Ala	4	4.6	4.1
Cys	2	—†	2.0‡
Val	1	1.0	1.0
Ile	1	0.9	1.0
Tyr	1	0.8	0.8
Phe	2	1.8	1.9
Lys	1	1.1	1.0
His	1	1.0	1.0

* Peptide resin hydrolyses usually give low values for Thr and Ser.
† Not determined.
‡ After performic acid oxidation and acid hydrolysis.

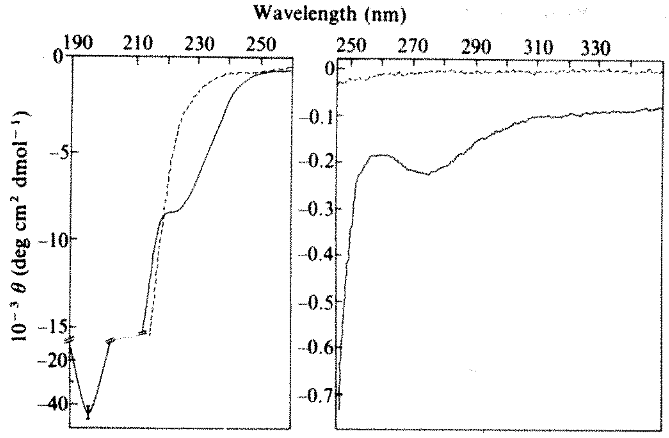

Fig. 6 CD spectrum of the 34-residue peptide. Measurements in the near and far UV region were carried out in a Dichrographe II with high sensitivity accessory and thermostated cuvette compartment using 0.1 mm to 10 mm cells. To determine molar concentrations absorption spectra were monitored in a Zeiss DMR 10 spectrophotometer. The peptide was dissolved in 0.01 M sodium citrate, *p*H 5.3 (solid line), and in 0.001 M sodium citrate–6 M guanidinium hydrochloride (dashed line), respectively.

dichroic absorption in the range of aromatic absorption at ~280 nm.

The 68-, 34-, and [Ala^{10}, Ala^{33}]-34-residue polypeptides showed similar optical properties in the aromatic region. In the far UV the presence or absence of cystine residues generated quantitative differences. A helical contribution was represented by a minimum at ~222 nm. Other characteristic features of protein CD spectra seemed to be shifted towards the UV region giving rise to a second minimum at 197 nm. The corresponding maximum was below 185 nm. A representative example is given in Fig. 6 (solid line) illustrating the overall CD profile of the 34-residue polypeptide. On addition of 6 M guanidinium hydrochloride (Fig. 6, dashed line) the CD bands in the aromatic absorption range and in the far UV region of the spectrum disappeared indicating a marked decrease in original secondary structure. Quantitative evaluation of helix, β-structure, and β-turn contents was not possible because of the specific interactions within the relatively small polypeptide molecule. Experiments dealing with solvent effects and ligand binding will be published elsewhere[22].

Discussion

Two problems must be solved before it becomes possible to design artificial proteins with novel properties for laboratory, medical and industrial use. First, assembly of the secondary structural elements to give the biologically active tertiary structure is at present only poorly understood; and second, chemical synthesis of proteins is still extremely difficult if not impossible. However, it is reasonable to assume that these obstacles will be overcome. Artificial proteins could also be produced by a genetic approach through expression of synthetic genes in bacteria[23]. An alternative route to artificial proteins is the thermal copolymerisation of amino acids yielding a mixture of proteinoids of varying composition and size. Such polymers have non-random amino acid composition[24] and are capable of catalysing chemical reactions such as hydrolysis, decarboxylation or amination[25]. Although the catalytic activities are weak, some thermal polyanhydro-α-amino acids do show substrate specificity and stereoselective action[25]. Isolation from the proteinoid mixture of single species possessing interesting enzymatic properties may be a formidable task and has not yet been reported.

Reproducible preparation of artificial proteins with distinct properties can probably best be achieved by a planned synthesis. An example for the latter approach is the synthesis by Chakravarty *et al.*[26] of an active lysozyme model consisting of only 10 amino acid residues. Based on the work of Kotelchuck and Scheraga[27], Chakravarty *et al.* designed a decapeptide that, aided by the polymeric nature of the substrates used, could adopt α-helical conformation displaying the essential unionised and ionised carboxyl groups in a suitable environment. This decapeptide showed weak glycosidase activity.

In the nucleic acid-binding polypeptide described here it is likely that stacking of the aromatic side chains with the bases of the nucleotides and hydrogen bonds in a hydrophobic environment contributed to the interaction energy. As the synthetic peptide was neutral, it possibly resembled in its properties several of the non-histone chromosomal proteins. The fact that cytidine phosphates were bound with relatively high specificity whereas interaction with the trinucleotide sequence around which the model has been built was weak, may have been due to differences between the proposed and the actual folding of the synthetic polypeptide. Preliminary CD measurements showed distinct CD bands in the aromatic and in the far UV absorption range that were extinguished in the presence of 6 M guanidinium hydrochloride. To determine secondary and tertiary structure more precisely we shall attempt crystallisation of the 34-residue polypeptide.

The synthetic nucleic acid-binding polypeptide also had ribonuclease activity with high preference for the cleavage of 3′,5′-phosphodiester bonds following 3′-linked cytidine residues. Dimerisation of the 34-residue polypeptide through formation of two intermolecular disulphide bonds improved the binding of cytidylic acid and enhanced the ribonuclease activity. This activity was not caused by microbial contamination since proteins such as insulin and bovine serum albumin were completely inactive in the nuclease assays after they had been passed through the same columns that were used for the purification of the synthetic polypeptides. As nucleases like RNase T$_1$, RNase A, or staphylococcal nuclease are among the shortest enzymes known, their action may be more easy to simulate by designed synthetic models than that of larger and more complex enzymes. Furthermore, the presence of a histidine residue in a position favourable for catalysis may have been essential for the nuclease activity of the 34-residue polypeptide because histidine residues are frequently found in the active site of hydrolytic enzymes.

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letters

X-ray and [Fe XIV] 5,303 Å emission from Puppis A supernova remnant

Hiroshi Itoh

Department of Astronomy, University of Kyoto, Sakyo-ku, Kyoto 606, Japan

Recently, Lucke *et al.*[1] found [Fe XIV] 5,303 Å emission from Puppis A to be much less intense than that expected from the X-ray data given by Zarnecki *et al.*[2]. We propose here that this discrepancy can be removed by taking into account the nonequilibrium state of ionisation within the X-ray source.

Interpreted under the assumption of ionisation equilibrium, the X-ray spectrum of Pup A consists of two components which have temperatures of 13 and 2.5×10^6 K (ref. 2). Therefore, if ionisation equilibrium indeed holds, the low-temperature plasma should emit [Fe XIV] 5,303 Å photons as observed in the solar corona. However, the measured [Fe XIV] emission[1] has been too weak to be in harmony with the volume emission measure[2] of the low-temperature plasma. Lucke *et al.*[1] have argued that the most plausible way of reducing this discrepancy to within experimental error is probably by assuming the maximum temperature of 3×10^6 K allowed by the X-ray data[2]. This interpretation, however, does not seem to be tenable, since ionisation equilibrium is unlikely to hold within the X-ray source. In fact, from a calculation[3] of time-dependent ionisation of oxygen for a post-shock temperature of 2.8×10^6 K, the time scale τ_I for ionisation relaxation within the low-temperature plasma is estimated to be $3\times10^4/n_e$ yr, where n_e ($\mathrm{cm^{-3}}$) is the post-shock electron density and has been estimated[4] to be about 8 $\mathrm{cm^{-3}}$; it can readily be shown that this value for n_e assures approximate pressure balance[5] between the low-temperature plasma and optical filaments[6,7]. We may then set $\tau_I\approx4\times10^3$ yr for the low-temperature plasma. On the other hand, a sure upper limit to the remnant's age, τ, can be obtained from the inequality, $\tau<r/v_b$, where r is the radius of Pup A which may be set equal to $7.3d$ pc (ref. 4), d (kpc) being the distance of Pup A, and v_b is the shock velocity of the blast wave propagating through the local intercloud medium with a density n_0 of about 0.1 H atom $\mathrm{cm^{-3}}$ or less[4]. Assuming approximate pressure balance between the low-temperature plasma and the blast wave, we may write $v_b\approx v_s(n_e/4n_0)^{1/2}$ (ref. 5), where v_s is the velocity of the shock producing the low-temperature plasma and is about $4.5\times10^7\ \mathrm{cm\ s^{-1}}$. Thus, we obtain $\tau<4\times10^3(n_0/0.1)^{1/2}\,d$ yr. Since $n_0\leqslant0.1$ H atom $\mathrm{cm^{-3}}$ and $d=1.2$–2.2 kpc (ref. 4), the condition for ionisation equilibrium, $\tau_I\ll\tau$, can hardly hold within the low-temperature plasma. A similar consideration for the high-temperature plasma which has a temperature of 13×10^6 K also leads to the same conclusion. Therefore, it seems more useful to examine a nonequilibrium ionisation model.

Recent calculations[8] of the time-dependent ionisation and X-ray emission of supernova remnants have shown that the nonequilibrium state of ionisation within the X-ray source results in an enhancement of the X-ray emission in the energy range below 1 keV, causing a two-component appearance of the X-ray spectrum. Although this result was obtained by assuming a uniform interstellar medium, it will hold qualitatively in the case of Pup A where the dominant X-ray source may be a shock-heated interstellar cloud[4]. The nonequilibrium ionisation model will predict much less [Fe XIV] emission than the equilibrium model, since in the former the X-ray emission in the sub-keV range is not ascribed to a plasma favourable to the [Fe XIV] emission.

We now evaluate the [Fe XIV] luminosity of the shock wave responsible for the X-ray emission from Pup A. After passing through the shock front, the electrons are rapidly heated and successively ionise the ions to higher stages[8]. The electron temperature of about 10^7 K inferred from the X-ray spectrum[2] indicates that the iron atoms would be almost stripped of their M-shell electrons under ionisation equilibrium[9]. Therefore, on its way to the equilibrium state, every iron atom passes through the stage of $\mathrm{Fe^{+13}}$. The average time interval during which an iron atom stays in this stage is $(n_e C)^{-1}$ seconds, where C ($\mathrm{cm^3\ s^{-1}}$) is the total coefficient for direct ionisation[10] and autoionisation[11] of $\mathrm{Fe^{+13}}$ by electron impact. Recombinations can be safely ignored in rapidly ionising plasmas. Also, because $(n_e C)^{-1}$ is of the order of only 10^9 s, changes in the electron temperature and density during this time interval can be ignored. Thus, after passing through the shock front, an iron atom emits $(n_e\alpha_e+n_p\alpha_p)\,h\nu/(n_eC)$ erg on the average at [Fe XIV], where n_p ($\mathrm{cm^{-3}}$) is the proton density, $h\nu$ (erg) is the photon energy, and α_e and α_p ($\mathrm{cm^3\ s^{-1}}$) are the coefficients for collisional excitation by electrons[12] and protons, respectively; for relatively high proton temperatures relevant to our case, α_p should be calculated from the cross-section data[13,14]. By assuming that the shock front has an area of 10^2S $\mathrm{pc^2}$ and is propagating at a speed of $10^8\,v\ \mathrm{cm\ s^{-1}}$ into a medium with a density of n_1 H atom $\mathrm{cm^{-3}}$ and an iron abundance of $4\times10^{-5}A$ in number relative to hydrogen[15], the [Fe XIV] luminosity of the shock wave may be written as

$$L=1.4\times10^{32}An_1vSR\ \mathrm{erg\ s^{-1}} \tag{1}$$

where

$$R=0.1\times[\alpha_e+(n_p/n_e)\alpha_p]/C \tag{2}$$

In Pup A, An_1v is probably near unity[1,4], while the value of S is uncertain. Referring to an estimate[4] that a fraction 0.1 of the total volume of Pup A is emitting in X-rays, we write $S=0.1^{2/3}\times4\pi\,(0.73d)^2\times s=1.4sd^2$, where s is a correction factor. The quantity R defined by equation (2) depends on the electron and proton temperatures in the [Fe XIV] emission region. If the electron and proton temperatures are quickly equilibrated just behind the shock front, we may adopt a single temperature of 10^7 K inferred from the X-ray spectrum[2]. In this one-fluid case, $R=0.81$ when $n_p/n_e=0.85$ (ref. 15), and equation (1) yields $L=1.6\times10^{32}An_1vsd^2\ \mathrm{erg\ s^{-1}}$. On the other hand, if the electrons are not heated significantly at the shock front compared with the protons, we should adopt a lower electron temperature and a higher proton temperature than 10^7 K since [Fe XIV] emission originates nearer to the shock front than X-ray emission. Inspection of the results of our recent calculations[8] gives electron temperatures of about 5×10^6 K for the $\mathrm{Fe^{+13}}$ region. The proton temperature need not be evaluated accurately, since α_p attains a maximum value of $4.1\times10^{-9}\ \mathrm{cm^3\ s^{-1}}$ at a temperature of 3.2×10^7 K and is very insensitive to the proton temperature in the range $(1–10)\times10^7$ K. We set $\alpha_p=4\times10^{-9}\ \mathrm{cm^3\ s^{-1}}$. In this two-fluid case, $R=1.9$, and equation (1) yields $L=3.7\times10^{32}An_1vsd^2\ \mathrm{erg\ s^{-1}}$. Thus, in both the one-fluid

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case and the two-fluid case, the nonequilibrium ionisation model predicts a [Fe XIV] luminosity that is of the same order as the observed[1,16] values of $(1\text{–}5)\times10^{32}\,d^2\,\mathrm{erg\,s^{-1}}$. Also, a similar calculation of [Fe X] 6,374 Å emission predicts that the [Fe X]/[Fe XIV] luminosity ratio is 0.14 in the one-fluid case or 0.098 in the two-fluid case, in accordance with an upper limit of 0.3 derived from the observation[1].

Thus the discrepancy found by Lucke *et al.*[1] between the X-ray and [Fe XIV] data on Pup A can be removed by the nonequilibrium ionisation model for the X-ray emission from this object. Note that the bulk of the observed [Fe XIV] emission need not be ascribed to a rapidly ionising plasma behind the shock front of the X-ray source. For instance, the brightest [Fe XIV] emission region observed near the edge of the radio shell seems, because of its high surface brightness, to have been produced by the interaction of the shock front with a density inhomogeneity[1], which contributes little to the X-ray emission. If that is the case, the [Fe XIV] emission from this region may be analysed under the assumption of ionisation equilibrium, since at the post-shock temperature of about 3×10^6 K the ionisation state of iron relaxes to the equilibrium much faster than that of oxygen[17]. Future observations with better spatial and spectral resolution will be important in clarifying the nature of the [Fe XIV] emission from Pup A.

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Hydrogen evolution from water induced by visible light mediated by redox catalysis

John Kiwi & Michael Grätzel

Institut de Chimie Physique, Ecole Polytechnique Fédérale, Lausanne, Switzerland

Noble metal dispersions are suitable for mediating light-induced hydrogen[1–5] and oxygen[6–8] evolution from water. We report here a dramatic improvement of the hydrogen production rate when very finely dispersed platinum is used as a mediator in reaction (1). The first observations of the dynamics of intervention of the Pt particles in the redox events are presented. A centrifuged colloidal Pt catalyst stabilised by polyvinyl alcohol showed exceptionally high activity in promoting hydrogen evolution from water via

$$2\mathrm{MV}^{+}+\mathrm{H_2O}\xrightarrow{\mathrm{Pt}}\mathrm{H_2}+2\mathrm{OH}^{-}+2\mathrm{MV}^{2+}\qquad(1)$$

where MV^{+} stands for reduced methylviologen. The latter is produced photochemically in aqueous solution containing $\mathrm{Ru(bipy)_3^{2+}}$ as a sensitiser and EDTA as an electron donor. At 10^{-3} M Pt the reoxidation of MV^{+} requires only 15 μs and leads to quantitative formation of $\mathrm{H_2}$.

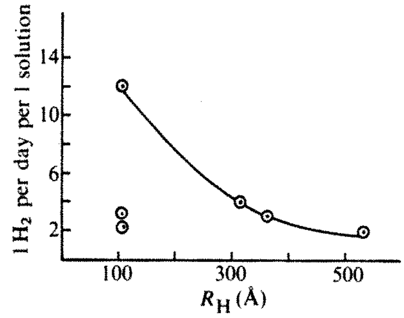

Fig. 1 Correlation for the observed yields for $\mathrm{H_2}$ in the system $\mathrm{Ru(bip)_3^{2+}}$ 4×10^{-5} M, MV^{2+} 2×10^{-3} M, EDTA 3×10^{-2} M at *p*H 5 and the radius of the catalyst particles used. Pt–PVA–60,000 catalysts centrifuged at 20,000 r.p.m. were used containing 3.5 mg Pt per 25 cm³ of solution.

The Pt/PVA catalyst was prepared by a procedure outlined by Nord[9]. By centrifuging this preparation at different speeds and for different times particle sizes between 530 and 110 Å are determined by quasi-elastic light scattering. The polymer PVA molecules are adsorbed on the surface of Pt metal through their less polar groups. The polymer also hydrates strongly in the presence of water due to its own OH groups. As long as the surface coverage is not complete, $\mathrm{H_2O}$ can penetrate to the Pt and the catalysis is mediated by a lyophobic system. In the case of particle size with radius 110 Å the size of the PVA particle on which the Pt is deposited is equal to the polymer PVA–60,000 indicating that there is a good dispersion of Pt on the polymer.

Steady-state irradiations were carried out with a 450 W xenon lamp through 400 nm cut-off filter and a water filter to eliminate IR radiation. Hydrogen was analysed by conventional GC with a molecular sieve 5 Å column. A detailed account of the light-induced events leading to irreversible reduction of (1'1-dimethyl-4,4'-bipyridinium^{+2}, abbreviated to MV^{+2}) to MV^{+} and oxidation of EDTA has been given elsewhere[3,4]. In the presence of colloidal Pt, MV^{+} reoxidises with simultaneous evolution of $\mathrm{H_2}$.

Figure 1 shows that a decrease of the particle radius from 500 to 100 Å leads to a drastic increase in the hydrogen evolution rate which is as high as 12 l per day per l solution for the smallest particle size. In the latter case, the bubbling of hydrogen gas which occurs when the solution is illuminated is readily seen. In Fig. 1 the common feature is the Pt content of the solutions of different particle size, namely 3.5 mg Pt per 25 cm³. Figure 1 also shows two extra points with 100 Å particle size but with low Pt levels; 0.35 mg Pt per 25 cm³ solution, the upper one, and 0.25 mg Pt per 25 cm³ solution, the lower one. The yields of $\mathrm{H_2}$ are 5 and 6 times, respectively, less than if 3.5 mg Pt per 25 cm³ solution is photolysed as shown in Fig. 1.

These observed size effects may be explained by the fact that the Pt particles intervene as microelectrodes[10–12] in the hydrogen evolution reaction (1). Electron transfer from MV^{+} to the Pt particles regulates these microelectrodes cathodically until $\mathrm{H_2}$ formation can take place. A smaller electrode size is advantageous both from the point of view of mass transport of the electroactive species as well as surface area per g of catalyst used. Note that in our experimental conditions the speed of electron turnover in the $\mathrm{MV}^{+}/\mathrm{MV}^{2+}$ relay—and not the photon flux or the kinetics of MV^{2+} photoreduction—is rate determining in the hydrogen generation process. The quantum yield for $\mathrm{H_2}$ formation was found to be half of that for MV^{2+} reduction, $\phi(\mathrm{H_2})=0.13$. This implies that the reoxidation of MV^{+} strictly follows the stoichiometry indicated in equation (1).

A further advantage of the centrifuged Pt/PVA is that the solutions remain completely transparent even at high catalyst concentration. This allows the direct study of the dynamics of the reaction of MV^{+} with Pt particles by laser photolysis. Figure 2*a–c* illustrates the temporal behaviour of the characteristic

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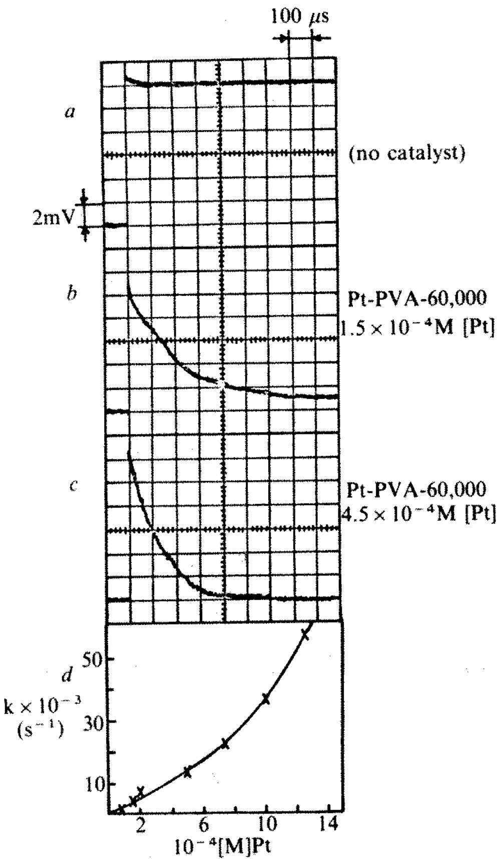


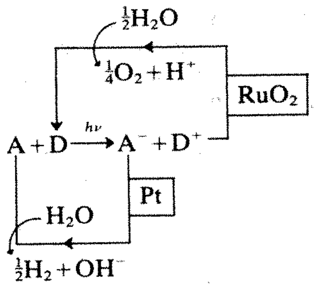
Fig. 2 Effect of the Pt–PVA catalyst concentration on the behaviour of MV^+ absorption at 600 nm. Representative oscilloscope traces are included in *a*, *b*, *c*. *d*, The rate constants for MV^+ decay are plotted against platinum concentration of the Pt–PVA catalyst used.

MV^+ absorbance at 602 nm in the absence and presence of catalyst. The upward deflection of the signal after the laser pulse is due to the formation of MV^+ via the photo redox process[3] sensitised by $Ru(bipy)_3^{2+}$. In the absence of catalyst the MV^+ absorption is stable, addition of colloidal Pt induces a decay of the signal the rate of which increases sharply with Pt concentration. Fitting the absorbance decay curves to an exponential time law gives the rate constants which are plotted as a function of Pt concentration in Fig. 2*d*. The curve is steeper than linear, indicating that the reaction order is greater than one with respect to the Pt concentration.

At a concentration of 10^{-4} M Pt in Pt–PVA–polymer, we observe a rate constant $k = 0.14 \times 10^3\ s^{-1}$. At the highest concentration of catalyst (12.5×10^{-4} M Pt content), the rate was $5.7 \times 10^4\ s^{-1}$. The lifetimes observed for MV^+ were shortened from about 7,000 μs to 15 μs, when the concentration of catalyst varied only by a factor of about 12.

The minimum concentration of platinum which gives efficient recycling of MV^+ under steady-state illumination is 0.5×10^{-4} M. The lowest useful concentration necessary to detect measurable quantities of photo-produced H_2 is 0.1×10^{-4} M in Pt (0.05 mg Pt per 25 cm^3). In this case, the evolution of H_2 observed was 0.010 l H_2 per day per l solution, but a noticeable amount of hydrogen gas bubbling out of solution could only be observed at 5 times the concentration of Pt necessary for the onset of H_2 evolution. Evidently, such a Pt content is sufficient to reoxidise the MV^+ rapidly enough to avoid a significant build up of MV^+ concentration. An onset value for the amount of Pt present seems necessary to obtain reasonable rates of H_2 evolution (see Fig. 1). The value for this onset is about 0.5 10^{-4} M Pt and the recycling at higher concentrations of Pt is shown in Fig. 2*b*, *c*.

These results are important for photo-redox splitting of water into hydrogen and oxygen:



it seems that by using a suitable catalyst, the conversion of A^- into A and simultaneous formation of hydrogen can be accomplished so rapidly that it can compete efficiently with the thermal backreaction. (In the photostationary state achieved under sunlight irradiation, the latter process, due to its bimolecular nature, should require at least several milliseconds.) Moreover, redox catalysts such as colloidal RuO_2 have been shown[7,8,13] to mediate very effectively the back conversion of D^+ into D under simultaneous production of oxygen. In fact, in a mixture of RuO_2 and Pt colloids serving as selective microelectrodes for the A/A^- and D^+/D redox couples, light-induced water splitting in a two-component system has recently been observed[13].

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Detection of elastic strain in an amorphous polymer by X-ray scattering

M. Pick*, R. Lovell & A. H. Windle

Department of Metallurgy and Materials Science,
University of Cambridge, Cambridge, UK

For crystalline solids, the displacement of diffraction peaks with applied elastic strain is the basis of one method of measuring internal stress[1,2]. The effect has been exploited for both metals[3] and crystalline polymers[4]. Hitherto, the diffuse character of diffraction peaks obtained from non-crystalline materials has discouraged similar measurements on them. Working with atactic polymethyl methacrylate (a-PMMA) as an example of non-crystalline polymer glasses (which can sustain elastic strains up to several per cent before plastic yield or fracture), a displacement of the most intense diffraction peak has been observed in response to applied elastic strain. This peak corresponds to intermolecular correlations in the polymer[5,6].

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* Present address: ICI Fibres, Hookstone Road, Harrogate, North Yorkshire, UK.

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X-ray diffraction scans from a-PMMA show three broad peaks of heights which decrease in order of increasing diffraction angle. Using CuKα radiation, the maxima are at 13°, 30° and 42° $2\theta_B$. (These angles correspond to Bragg spacings of 6.8, 3.0 and 2.2Å respectively, which, of course, for a non-crystalline material have limited meaning.) Orientation of the PMMA glass by plastic deformation demonstrates that the major component of the first peak is equatorial and represents intermolecular correlations, whereas the 30° and 42° peaks concentrate on the meridian and are intramolecular in origin[5,6].

Dumbbell shaped tensile specimens, cut from 1 mm thick sheet (ICI Perspex), were stressed at room temperature and held at constant elastic strain. The maximum extension obtained before fracture was ~11%. Diffraction scans of the strained specimens were obtained using a standard transmission diffractometer. Examples for a specimen strained to ~8.8%, with the scattering vector first parallel to and then perpendicular to the tensile axis, are shown in Fig. 1. Also included for comparison is the diffraction scan for an undeformed specimen. As can be seen, the principal effect is a strain-induced displacement of the first peak. It moves to a lower angle when the scattering vector is parallel to the tensile axis, and to a higher angle when the vector is normal to the axis.

The fact that the positions and intensities of the second and third peaks, which are sensitive to molecular conformation[6], are not significantly affected by elastic strain indicates that for this polymer the underlying conformation does not change in response to the applied stress. There is also no suggestion of orientation of the molecular axes as a result of elastic strain, although this commences once the polymer yields and leads to an intensification of the second and third diffuse peaks on the meridian[7].

Figure 2 shows the displacement of the first peak as a function of applied elastic strain. The right hand ordinate expresses the percentage change in interchain spacing as determined by the change in cosec θ_B.

For a crystalline solid the most appropriate simple model would be one in which the material deforms affinely, that is the chain segments move as though embedded in a continuum. Such a model predicts a change in the interchain spacing equal to the applied strain. However, for PMMA the X-ray measurements of the microscopic strain give values which, although in the same sense, are much smaller than the strains applied. A tensile strain of 10% produces an increase in interchain spacing of only 1.7%, whereas the associated lateral compressive strain which, for PMMA with a poissonian ratio of 0.39, will be 3.9% apparently corresponds to a decrease in interchain spacing of 1.1%. The microscopic strains are thus, 17% and 28% respectively, of the applied strain.

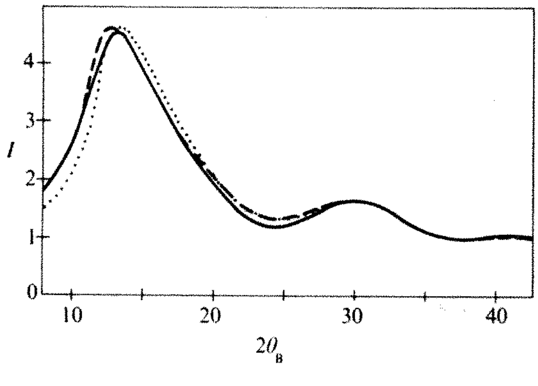

Fig. 1 Comparison of diffracted intensities for scans: dashed line, scattering vector parallel to the applied tensile stress; dotted line, perpendicular to the stress; solid line, for the unstressed specimen. The applied tensile elastic strain was 8.8%.

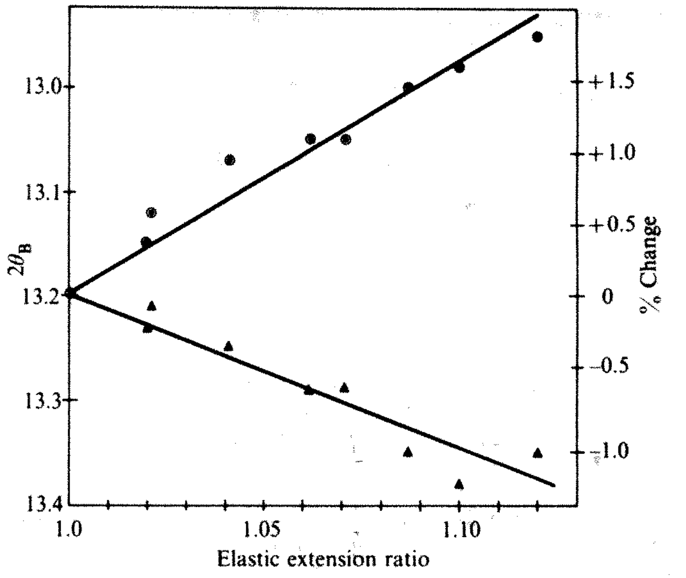

Fig. 2 The change in position of the first broad diffraction peak as a function of elastic strain. ⊙, Scattering vector parallel to the applied tensile stress; ▲, perpendicular. The right-hand ordinate is calibrated in terms of the change in apparent chain spacing.

The other simple model is one in which the microscopic strain is solely due to the increase in volume of the material (caused by the hydrostatic component of the tensile strain). While this predicts a change in interchain spacing of approximately the correct amount (10% tensile strain giving ~1% increase in interchain spacing for PMMA), it is unable to account for the fact that the change normal to the tensile axis is a decrease.

These discrepancies suggest that a more appropriate model may be a non-affine one in which the distance between nearest-neighbour chains remains essentially constant. For PMMA, which has a fairly rigid backbone, such contacts may involve some correlation of segmental orientation. However, even without such correlation, the distance between nearest-neighbours initially in contact is likely to change less under stress than will the distance between near-neighbours which are not in contact. Since it is the nearest-neighbour contacts which have a dominant influence on the position of the intermolecular diffraction peak, such a model would explain the reduced sensitivity of peak position to applied strain. At the same time, the volume increase and macroscopic strain would be accounted for by a change in the number and arrangement of the near-neighbour chains.

The fact that there is still no satisfactory model to describe molecular packing in non-crystalline polymers means that these arguments cannot be developed further and quantified. Also, any detailed understanding will have to take into account the thermally activated (anelastic) component of the applied strain which is associated with changes of local molecular orientation, or of sidegroup geometry, which themselves will influence packing.

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Radiative cooling near the mesopause

D. C. Allen*, J. D. Haigh†, J. T. Houghton† & C. J. S. M. Simpson*

* Physical Chemistry Laboratory, Oxford University
† Department of Atmospheric Physics, Clarendon Laboratory, Oxford University, UK

The heat budget of the region of the upper atmosphere near the mesopause at ~85 km is determined by a balance between radiative, photochemical and dynamical effects leading to a very cold polar mesopause in the summer and a comparatively warm mesopause in the winter. There is a temperature minimum at the mesopause primarily because of the strong radiative cooling which occurs due to thermal emission by carbon dioxide in its ν_2 vibrational band at 15 μm wavelength. Above 80 km this band is no longer in local thermodynamic equilibrium (LTE) so the amount of cooling depends critically on the rate at which CO_2 molecules are excited or relaxed by collision. Any estimate of radiative cooling for this region of the atmosphere, therefore, relies on knowledge of the collisional relaxation time, τ. The importance of the temperature dependence of τ was pointed out by Houghton[1]. Previous calculations used a wide range of values because no direct measurements of τ had been made in the appropriate temperature range. We show here that the effect on cooling rate calculations of using values of τ measured at temperatures down to 175 K. We also estimate a radiative relaxation time for the atmosphere near the mesopause and are able to draw conclusions as to the natural lifetime of any temperature perturbation at this altitude.

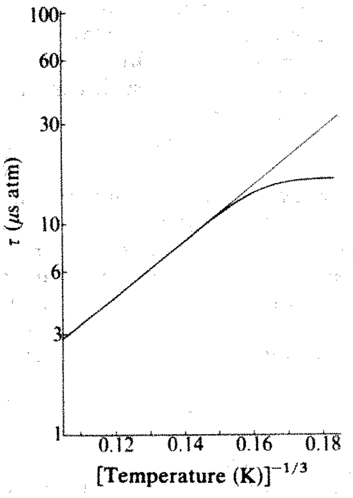

Fig. 1 The relaxation time of the ν_2 vibration of CO_2 due to CO_2–N_2 collisions. The thin line shows a linear extrapolation of the higher temperature measurement.

The CO_2 molecules may be excited into the ν_2 state by the N_2, O_2 and O atoms present. Water is a very effective collision partner, but in the upper atmosphere its effect is negligible owing to its very low concentration. In previous calculations of cooling rates[1–4], values of the relaxation time, τ, at 1 atm between 2 and 50 μs have been used.

The reason for this spread was because of the difficulties in making reliable estimates of τ. A scatter of at least ±25% existed in the older data at 300 K[4] and it was not known whether the nearly linear plots of log τ against $T^{-1/3}$ could be extrapolated down to 180 K. For instance, on the basis of data for the relaxation time of CO_2 by CO_2 and by Ar taken down to 150 K[6], perhaps the best estimate of the relaxation time of CO_2 by air at 210 K to be made up to the present was 22 μs (ref. 7).

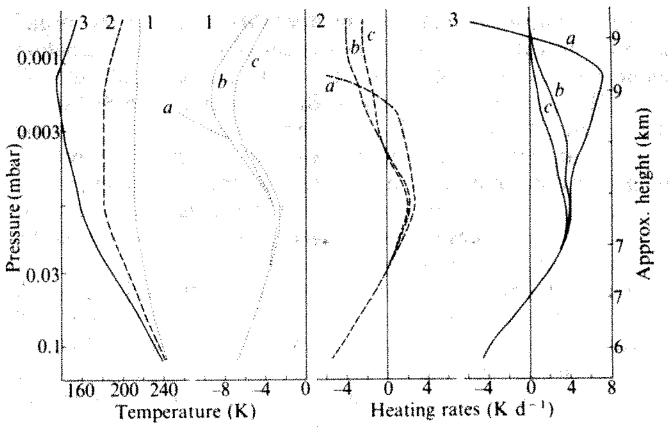

Fig. 2 Temperature profiles for (1) January at latitude 70 °N; (2) USA 1962 Standard Atmosphere; (3) December at latitude 70 °S. And the corresponding heating rates assuming (*a*) local thermodynamic equilibrium, (*b*) the new measurements of τ, (*c*) τ values from extrapolation of measurements at higher temperatures.

Figure 1 shows the results of recent laser fluorescence measurements of the relaxation time of the ν_2 vibration of CO_2 due to CO_2–N_2 collisions. Further details of these measurements are given by Allen[8]. They are the first direct measurements over the range of temperature appropriate to the mesopause region, and show much less dependence on temperature at temperatures below 250 K than would be expected from an extrapolation of the higher temperature data. On the basis of the work of Simpson *et al.*[7] we anticipate that the relaxation time for CO_2 by O_2 will be about 20% faster than for CO_2 by N_2. For air at 180 K this gives $\tau = 15 \pm 2$ μs at 1 atm, that is, 30% less than the previous best estimate.

In Fig. 2 the effects of different values of τ on the radiative divergence near the mesopause are calculated using the Curtis matrix method as described by Williams[4] and Houghton[9]. This method allows for transfer of radiation between different levels of the atmosphere and for the breakdown of local thermodynamic equilibrium: the transmission function incorporates the Curtis–Godson approximation, a constant diffusivity factor and a band model of independent lines. Note that an additional radiative cooling of ~2 K d^{-1} arises from the difference between

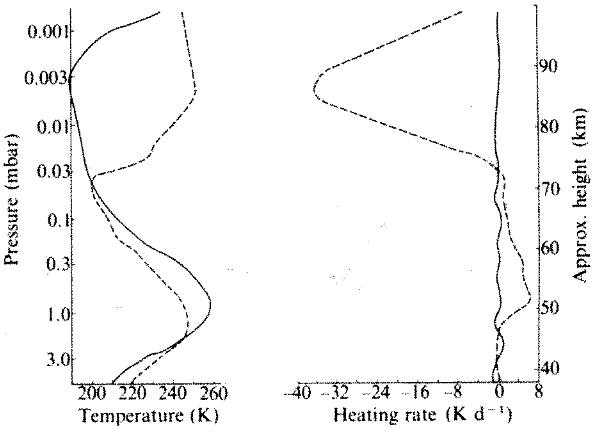

Fig. 3 Solid lines show approximate radiative equilibrium temperature profile calculated for latitude 30 °N in January and its corresponding heating rate. Dashed lines show an extreme temperature profile measured from a rocket[8] and its corresponding heating rate.

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using straight extrapolation and using the latest values. In the absence of other heating mechanisms this would have to be balanced by dynamical heating due to an increase in downwards motion of about 200 m d^{-1}—a very significant consideration in the context of the mesospheric circulation.

Another quantity which is important in understanding the structure of the mesosphere especially in perturbed conditions is the time constant with which radiation processes tend to return a perturbed atmosphere to the mean state. Figure 3 shows a radiative equilibrium temperature profile calculated for latitude 30 °N in January using the Curtis matrix method, with the new τ values, and for the 15 μm CO$_2$ cooling and an 'exact' frequency dependent calculation for the heating due to absorption of solar UV radiation by ozone and molecular oxygen. Also shown is a rather extreme temperature profile similar to one measured on 4 January 1976 by some rockets[10]. Other data in the same series suggest that this profile persisted for some while being part of a wavelike disturbance with period 7 or 12 days. The net radiative heating rate was calculated for the latter profile using the methods referred to above. It shows a cooling of 36 K d^{-1} where the temperature deviates by 60 K indicating that the radiative time constant at 85 km altitude is of the order of 2 days. Perturbations of the mesospheric temperature, therefore, are very strongly damped and cannot persist for periods of longer than a few days unless they are continuously forced.

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Intraplate earthquake swarms in Greenland and adjacent continental regions

Søren Gregersen

Geodaetisk Institut, Gamlehave Alle 22, DK-2920 Charlottenlund, Denmark

Earthquake swarms, consisting of many earthquakes of nearly equal magnitude within a small area, often occur in areas of recent or current volcanic or tectonic activity[1]. But earthquake swarms can also be recognised in some typical intraplate areas in northern Canada[2], Norway and Greenland. A swarm of earthquakes started in Meløy in November 1978, remarkable because no earthquake swarms in Norway[3] have been documented previously. The Meløy swarm occurred near the Norwegian coast, on one side of the Norwegian/Greenland Sea. A few earthquake swarms have occurred also in Greenland, near the east coast, by the Norwegian/Greenland Sea, and near the north coast, by the Arctic Ocean as have most of the swarms in northern Canada[2,4]. Some common features of the earthquake swarms in Greenland, Norway and northern Canada are indicated here.

The earthquake swarms in Greenland have occurred in areas with a tectonic setting and an earthquake activity similar to that of Norway. Figure 1 shows two time sequences of earthquakes within small areas. As none of the earthquakes is the main event in either sequence, the sequences are termed earthquake swarms. The largest earthquakes of the swarm in eastern Greenland had magnitude 2.7 according to the Nuttli scale[5]. The smallest earthquakes of the swarm, which can be identified as having occurred in the same source area, had magnitude 1.9. A simple linear relationship between the magnitude and the logarithm of the number of earthquakes in each magnitude range could not be determined reliably—no b-value could be found for the earthquake swarm. Altogether the energy release in the swarm corresponds to the energy release of one single earthquake of magnitude a little over 3 (a small event).

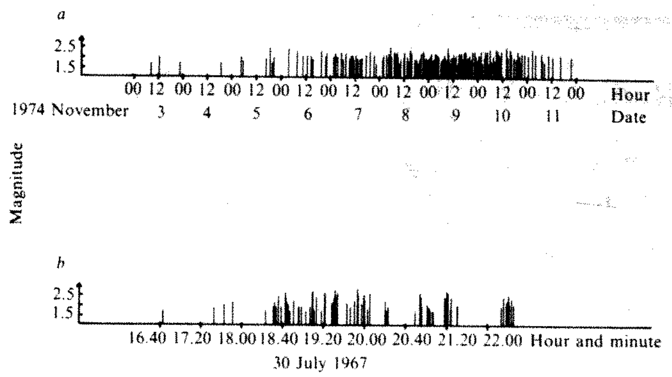

Fig. 1 Time sequences of the earthquake swarms in eastern (*a*) and northern (*b*) Greenland. Magnitudes are based only on readings from the seismological stations DAG and ALE respectively. No earthquakes occur at the swarm locations either immediately before or after the illustrated time intervals.

The earthquake swarm in northern Greenland lasted a shorter time than that in eastern Greenland. (The time scales of Fig. 1 *a* and *b* are different; 2 min for the sequence in northern Greenland correspond to 1 h for the sequence in eastern Greenland.)

The largest earthquake of the swarm in northern Greenland had magnitude 2.9 according to the Nuttli scale[5]. The smallest earthquakes, which can be clearly identified to have happened in the same source area, had magnitudes 1.8. The b-value for the earthquake swarm is around 1.0 with a large uncertainty, because the magnitude range is small. The energy release in the swarm corresponds to the energy release of a single earthquake of magnitude a little over 3, which is a small event and similar to that in eastern Greenland. In the swarm in eastern Greenland ~160 earthquakes have been distinguished, while in the swarm in northern Greenland 80 earthquakes have been found.

In eastern Greenland the earthquake swarm location around 73.5 ° N 20° W (Fig. 2) is based on seismograph records of the 25 largest earthquakes of the swarm from two seismological stations in Danmarkshavn (DAG) and in Kap Tobin (KTG). The uncertainty in the location of the swarm is 100 km in an east–west direction, while it is 25 km in a north–south direction. The earthquake swarm is in an area where neither single earthquakes nor earthquake sequences are known to have happened previously. The smaller earthquakes of the swarm have the same recorded signature at station Danmarkshavn (DAG) as the larger earthquakes, but they cannot be distinguished at the less sensitive station Kap Tobin (KTG). None of the earthquakes were recorded at other seismological stations.

The calculation of the location of the earthquake source area in northern Greenland 81.5° N 49° W (ref. 4) (Fig. 2) is based on records from the seismological stations Alert (ALE), Inge Lehmann (ILG) and Nord (NOR). Not all of the earthquakes could be distinguished at station Nord. Earthquake sequences have been observed at the station Alert several times before, as well as after, the sequence in Fig. 1, at similar distances, presumably near the same location. The ILG station operated for only 1 yr and only the abovementioned earthquake sequence occurred during that time. The earthquakes were not recorded at other seismological stations.

In northern Canada earthquake swarms are quite common[2]. All of the swarm locations there are plotted in Fig. 2. In Canada, Greenland and Norway the swarms are situated a long way from

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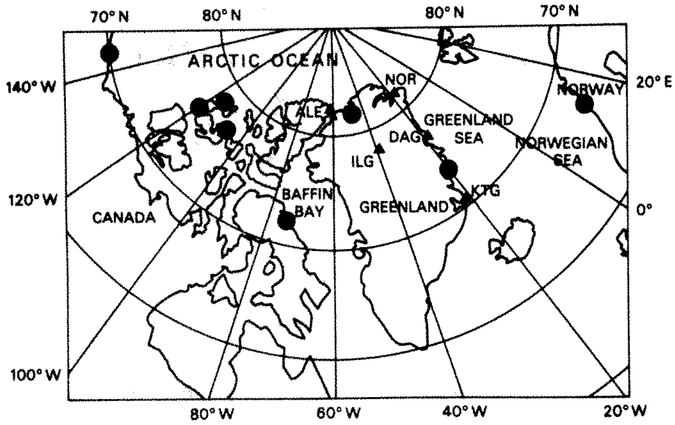

Fig. 2 Map with the recognised intraplate earthquake swarms (●). For the continental regions adjacent to Greenland the locations of the earthquake swarms are from published sources[2–4,6–10]. ▲, Seismological stations, mentioned in the text, with a three-letter code.

present plate boundaries. The swarms in Norway and in eastern Greenland are close to the passive continental margins of the Norwegian/Greenland Sea, in the middle of which an active mid-ocean ridge is located[11]. The swarms in northern Greenland and northern Canada[2,4] are located close to the passive margins of the Arctic Ocean and Baffin Bay. In the Arctic Ocean there is an active mid-ocean ridge[2]. In Baffin Bay there is no active mid-ocean ridge; the active spreading ceased >40 Myr ago[12]. In all of the swarm locations there are basins of sediments close to the continental margins[3,4,13,14]. The swarm activities are close to these sediment basins, whether these are of Palaeozoic ages as in northern Greenland or of Mesozoic ages as in eastern Greenland. These swarm locations are at the edges of continental areas which since the end of the last ice age (~10,000 yr ago) have been exposed to considerable uplift. The earthquakes in the swarms are found to be shallow in the cases where they could be determined[3,4,6] so the earthquake swarms presumably occur in the upper crust.

The swarms did not occur in the usual locations of earthquake swarm activity, which are areas of current or recent volcanic or tectonic activity[1]. They occurred far from interplate boundaries, so they may be classified as intraplate earthquake swarms. Sykes[15] noted that many intraplate earthquakes occur along old zones of weakness near continental margins and the same interpretation is reached from the present results. The earthquake swarms occurred along continental margins where the structures in and around the old sedimentary basins are the zones of weakness. The geological ages of the sedimentary basins are of little importance. The tectonic stresses which give rise to the intraplate earthquakes may be those connected with the present plate tectonics motion[15], but a special source of stresses in Greenland and the adjacent continental regions is the considerable uplift of these regions since the last ice age.

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Radiocarbon chronology of late Pleistocene Konya lake, Turkey

Neil Roberts*, Oğuz Erol†, Titus de Meester‡ & Hans-Peter Uerpmann§

* Department of Geography, University College London, Gower Street, London WC1, UK
† Coğrafya Fakültesi, Ankara Universitesi, Ankara, Turkey
‡ Landbouwhogeschool, Wageningen, The Netherlands
§ Institut für Urgeschichte, Universität Tübingen, Tübingen, FRG

The Konya basin in south central Turkey, which is now largely dry, was formerly occupied by an extensive lake[1,2]. Although the lake is generally considered to be of glacial age, its precise chronology has remained uncertain[3]. We report here preliminary ^{14}C dates on shells which suggest that lake level attained its last maximum 23,000–17,000 yr ago. We further propose that its subsequent retreat reflects a late glacial climate that was dry as well as cold.

The Konya plain is an area of internal drainage lying ~1,000 m above m.s.l. on the southern edge of the Anatolian plateau (Fig. 1). Rivers enter the basin from the Toros (Taurus) mountains to the south and west, and eventually discharge into small lakes at Ak gölü and Hotamıs and marshes at Aslım and Hamidiye. The rest of the plain is now dry. Shorelines of the former lake can easily be traced from eroded cliff-lines and depositional features such as beaches and spits, which do not seem to have been subsequently affected by folding or faulting. The lake covered ~4,340 km^2 at its last maximum, but the depth of water did not exceed 30 m and at most places was <15 m. The Konya lake was thus larger but much shallower than the contemporary Lisan Lake in the Jordan rift[4].

Most of the ^{14}C samples discussed here came from shell beds in sand and gravel deposits of shoreline features. The Main Series of dates were from samples taken at elevations between 1,004 and 1,012 m, and those in the Upper and Lower Series were on samples from higher and lower elevations respectively (Table 1). All dates were determined on shell, and in all but two cases the species involved was *Dreissena*, a small aragonitic bivalve which prefers fresh or brackish, open-water conditions[5]. The two exceptions, which date a minor lake transgression (Lower Series), were on *Lymnaea*, which was one element in a molluscan assemblage indicative of shallow, stagnant water[5,6].

Contamination by young carbon is the major problem in the ^{14}C dating of shells older than 10,000 yr (ref. 7). Several shells in the Konya samples were found to have a secondary coating of calcite which had formed by percolating meteoric water after the retreat of the lake. The distorting effect of this on radiocarbon age is seen clearly in a sample from Dervişin Hanı, where the inner and outer shell fractions gave ages of 17,610 ± 160 and 11,540 ± 120 yr BP respectively, the latter age having been depressed by the secondary calcite cement (Table 1). One of the Kılbasan samples (10.a.i.) was also found to contain up to 8% calcite, and its age of 14,660 ± 155 yr BP should therefore be considered as a minimum.

Other samples were analysed by X-ray diffraction and optical and scanning electron microscopy, and were hand-sorted to remove contaminated specimens before submission to the radiocarbon laboratory. This procedure allowed elimination of secondary calcite at levels above 1–2%. The resulting improvement can be seen in sample 2.a.i., also from Kılbasan, where the discrepancy between the ages of inner and outer shell fractions was greatly reduced. But because of these problems and of possible uptake of 'old' carbon, it is felt that a 10% margin of error should be applied to most of the ^{14}C dates listed here.

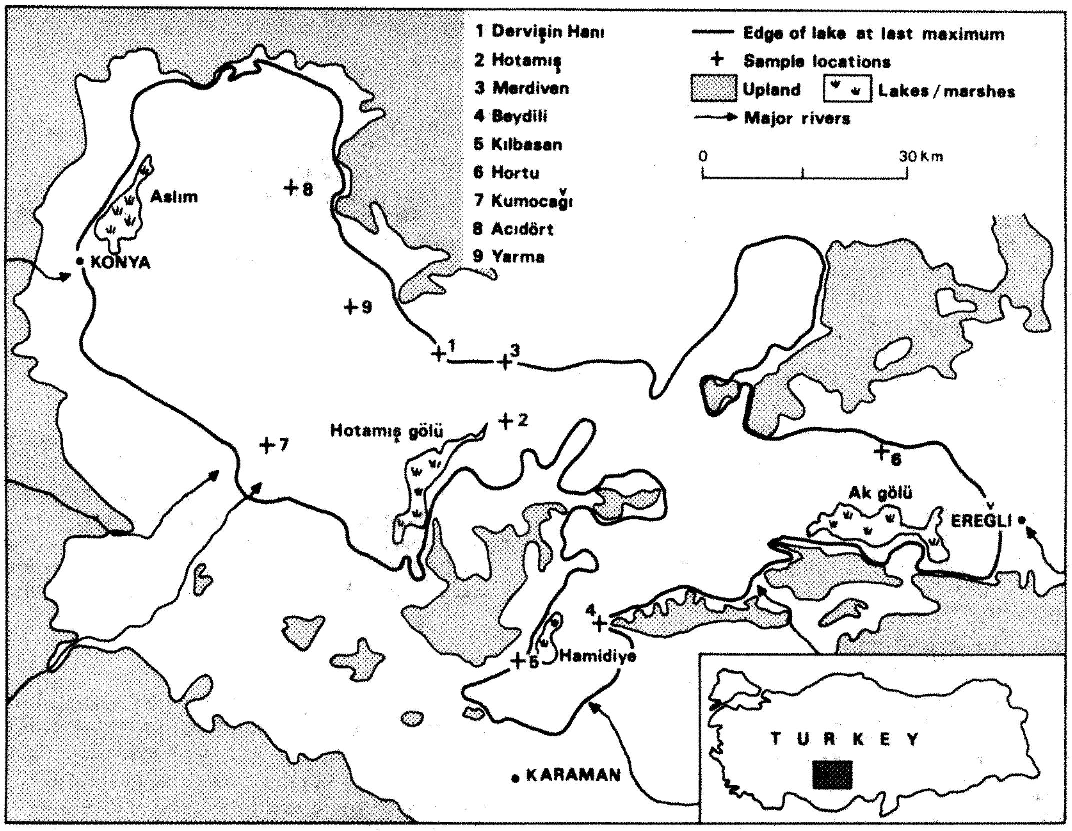

Fig. 1 Location of sample sites in Konya basin.

The radiocarbon dates are, nonetheless, consistent in suggesting that the last major phase of high lake levels in the Konya basin occurred between 23,000 and 17,000 yr BP. The lake had existed in the period immediately before this, but was at a low level. On the other hand, after 17,000 yr BP during the late glacial stage, the basin seems to have been largely dry. A minor re-advance in isolated secondary depressions between 12,000 and 11,000 yr BP may have been caused by glacial meltwater and by overflows from a higher lake system in the Beyşehir–Suğla basin. Archaeological mounds or hüyüks lie on the floor of the Konya basin and thus post-date the last lacustral interval. The oldest excavated hüyüks are Can Hasan III (with ^{14}C dates back to 8,600 yr BP)[8] and Çatal hüyük (dated between 8,200 and 7,500 yr BP)[9]. The fauna from Can Hasan III indicates generally dry, steppic conditions in the Karaman sub-basin around 8,500 yr BP (S. Payne, personal communication). The Konya basin has therefore been dry for most of the Holocene, although there is evidence for more extensive and permanent flooding in the Konya–Çumra area during the mid-Holocene[3].

The proposed late Pleistocene lake level sequence from Konya is in general agreement with those from other Middle Eastern basins, such as Lakes Lisan[4] and Mundafan[10], where the last high-stands occurred before $16\text{–}17 \times 10^3$ yr ago. It also differs markedly from the sequence in the south-west US, where many palaeo-lakes were at high levels between 17,000 and 10,000 yr BP (ref. 11). The early Holocene lacustral episode well

Table 1 Radiocarbon dates for the Konya lake

Field site	Location	Laboratory no.	^{14}C age (yr BP)	Sample no. and comments
Upper Series				
Merdiven	37°44′ N, 33°13′ E	GrN 6015	32,350±410	
Main Series				
Kılbasan	37°21′ N, 33°13′ E	UM 1638	22,850±370	2 a i inner shell fraction
Kılbasan	37°21′ N, 33°13′ E	UM 1637	20,800±190	2.a.i. outer shell fraction
Hortu	37°37′ N, 33°49′ E	GrN 5728	21,800±455	shells from white lake marl
Beydili	37°23′ N, 33°22′ E	UM 1639	$21{,}790^{+700}_{-650}$	8.b.i.
Beydili	37°23′ N, 33°22′ E	UM 1579	19,510±450	8.b.i. (rpt.)
Kumocağı	37°37′ N, 32°48′ E	GrN 6014	21,370±215	
Hotamış	37°39′ N, 33°11′ E	GrN 5729	20,700±450	shells from white lake marl
Kılbasan	37°21′ N, 33°13′ E	UM 1568	18,915±325	2.b.ii.
(Kılbasan	37°21′ N, 33°13′ E	UM 1577	14,660±155)	10.a.i. (minimum age)
Dervişin Hanı	37°45′ N, 33°05′ E	H 4181–3412	17,610±160	Ky 1b inner shell fraction
(Dervişin Hanı	37°45′ N, 33°05′ E	H 4181–3411	11,540±120)	Ky 1a outer shell fraction (minimum age)
Lower Series				
Yarma	37°48′ N, 32°58′ E	GrN 6016	12,010±65	
Acıdört	37°58′ N, 32°52′ E	GrN 5841	10,950±65	shells from dark organic marl

All dates were calculated using the 5,568 half life, and are normalised for ^{13}C fractionation to $\delta^{13}C = -25‰$ relative to PDB. Samples listed in brackets have suffered secondary contamination, and their ^{14}C ages are taken to be minima.

documented for much of Africa[12] and Arabia[10] is not recorded in the Konya basin.

The shallowness of the Konya lake and its disappearance during the post-glacial rise in temperature both point to the critical role of evaporation in the water balance of the lake. As there is no evidence of major temperature changes associated with the main lake retreat, one must conclude that this regression was a product of climatic aridity. A decline in precipitation during the late glacial is also indicated by very low levels of arboreal pollen in cores from Söğüt and the Ghab[13]. It thus seems that not only the tropics but also the extra-glacial mid-latitudes were subject to major variations in precipitation during the glacial Pleistocene. These variations seem, moreover, to have been out of phase with the global palaeotemperature changes indicated by deep-sea cores[14]. Work now in progress is intended to relate the Konya sedimentary sequence to palynological and archaeological data, and to elucidate the palaeohydrology of the basin.

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Chlorophyll and nitrate fine structure in the southeastern Bering Sea shelf break front

R. L. Iverson*, T. E. Whitledge† & J. J. Goering‡

* Department of Oceanography, Florida State University, Tallahassee, Florida 32306
† Division of Oceanographic Science, Brookhaven National Laboratory, Upton, New York 11973
‡ Institute of Marine Science, University of Alaska, Fairbanks, Alaska

Ocean fronts of considerable spatial extent and temporal persistence have been described for the shelf break region along the North American Atlantic coast[1–3] and along the eastern Bering Sea shelf[4]. Intensive fishing activity in the vicinity of the shelf break front along the mid and north Atlantic coast suggests that high biological activity is associated with the front[5,6]. Primary productivity and chlorophyll standing crop are enhanced along the Scotian shelf break front as a consequence of vertical transport of nutrients to the photic zone[7,8]. Although physical data suggest that shelf break fronts persist for periods of from weeks to years, space and time scales associated with biological activity in shelf break fronts are not well known. We report here the spatial scales and fine structure of chlorophyll and nitrate distribution in near-surface waters of the shelf break front in the southeastern Bering Sea. Data collected on several cruises during an investigation of the processes and resources of the Bering Sea shelf (PROBES) allow a first order estimate of the time scales of persistence of nutrient and phytoplankton dynamics associated with the shelf break front. Properties associated with an extensive middle shelf front inshore of the shelf break front are reported elsewhere[9].

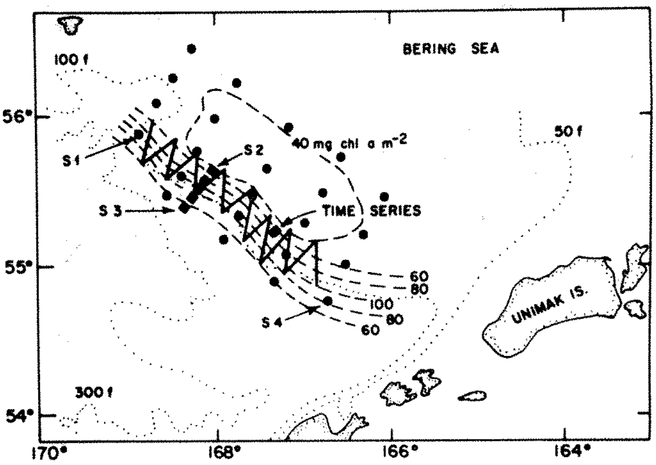

Fig. 1 PROBES study area in the southeastern Bering Sea. Contours of vertically integrated chlorophyll (mg m^{-2}) were prepared from data taken at hydrocast stations (●). The mapping track is indicated by the solid lines. The time series station location lies near the arrowhead. SI is station 3002, S2 is station 116, S3 is station 120, and S4 is station 3038.

CTD data taken at hydrocast stations (Fig. 1) were processed with a digital computer aboard the RV Thomas G. Thompson. The shelf break front was evident near station 120 in both density and salinity data (Fig. 2). The shelf break front is characterised by a salinity gradient which extends about 1,000 km along the continental shelf of the eastern Bering Sea[4].

Chlorophyll values were obtained by *in vivo* fluorometric measurement of phytoplankton pigments in water obtained from the sea chest of the RV Thomas G. Thompson ($z = 3$ m). Continuous *in vivo* chlorophyll values measured with a fluorometer (Turner Designs) were calibrated against extracted fluorescence chlorophyll values for water samples taken every 30 min from the exhaust side of the fluorometer ($r^2 = 0.91$). Nutrient concentrations were measured continuously with an Autoanalyzer for water samples taken from the sea chest line[10]. One-minute interval samples were taken from the detectors

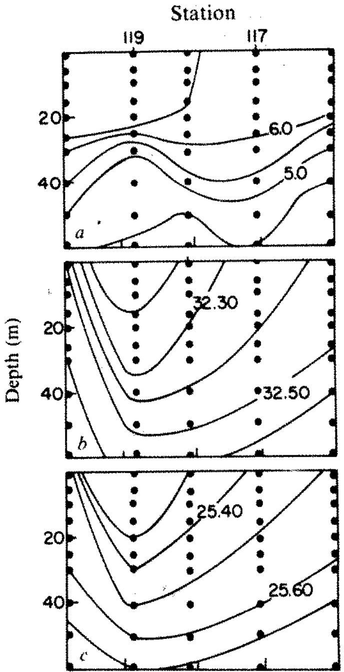

Fig. 2 Sections of *a*, temperature (°C); *b* salinity (‰); and *c*, density (σ_t) across the shelf break front, 11 June 1978.

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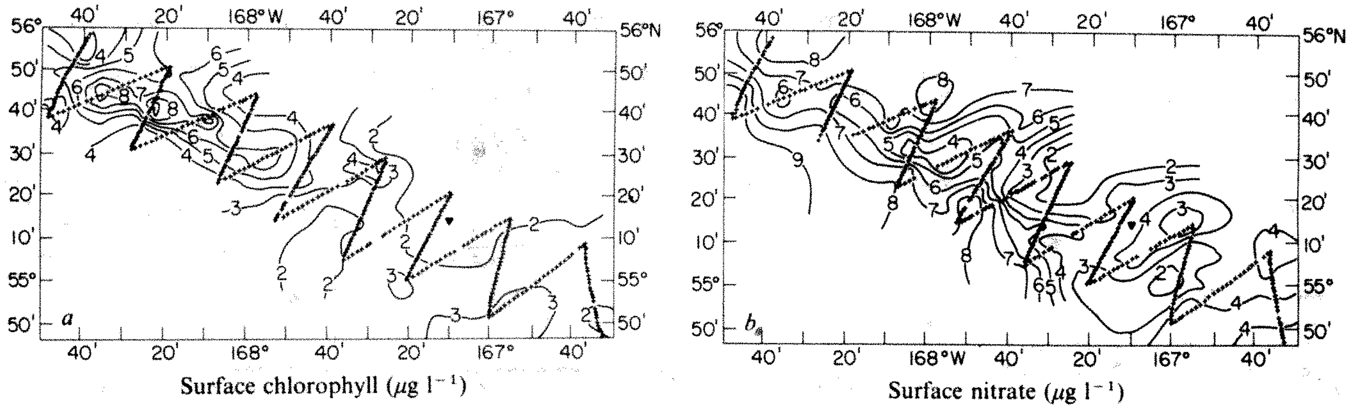
56°
50'
40'
30'
20'
10'
55°
50'
a
40'
20'
168°W
167°
56°N
Surface chlorophyll ($\mu g\ l^{-1}$)
b
Surface nitrate ($\mu g\ l^{-1}$)

Fig. 3 *a*, Map of chlorophyll concentration ($\mu g\ l^{-1}$) at 3 m depth on 26 May 1978. The time series station was located at the triangle. *b*, Map of nitrate concentration (μg atoms of NO_3 per l of N) and 3 m depth on 26 May 1978.

over a 36-h mapping track (Fig. 1). Five-minute averages of the samples were contoured on board ship with a digital computer to produce maps of chlorophyll and nutrient concentrations. Nutrient and chlorophyll concentrations were measured for water samples taken from Niskin bottles tripped at various depths at selected stations. Uptake velocities of nitrate and ammonium were determined for phytoplankton communities using a ^{15}N isotope tracer technique[11]. Samples were taken periodically for microscopic examination of phytoplankton.

Vertically integrated chlorophyll values obtained from extracted fluorescence measurements for discrete water samples obtained from hydrocasts were contoured with a digital computer program. Chlorophyll was distributed along the shelf break in a band about 30 km wide with a maximum in the region of the 100 fathom isobath; integrated chlorophyll values decreased by about half on either side of the frontal maximum (Fig. 1). The front moves with the tide, with an estimated maximum speed[12] of 50 cm s^{-1}, which explains the difference in location of the front in the physical data (Fig. 2) and the location of the chlorophyll maximum obtained by contouring data taken at various stages of the tide.

A surface mapping track was designed to bracket the 100 fathom isobath from the Aleutian Island chain north to the Pribilof Islands. A fine structure map prepared from data collected on this track suggests the chlorophyll maximum was ~15 km wide with chlorophyll values generally larger towards the Pribilof Islands (Fig. 3*a*). The map track covered a horizontal distance of about 170 km along the shelf break. Although the gradients across the front were evident in the chlorophyll field along the complete length of the map, structure in the near-surface chlorophyll field was most distinct from background chlorophyll in the northern 100 km. Structure in the nitrate field was not as detailed as structure in the chlorophyll field; however, local minima in nitrate concentration generally coincided with local maxima in chlorophyll concentration (Fig. 3*b*). A subsurface chlorophyll maximum present in the southern portion of the transect (Fig. 4) was not observed in the mapping data set because of the 3-m sampling depth. Silicate and phosphate concentrations did not exhibit the structure observed in the near-surface nitrate map but the general trends were similar with higher values to the north and lower values near the Aleutian Island chain.

A time series station was occupied within the shelf break chlorophyll maximum region in early May before mapping the front in late May. The vertical distribution of chlorophyll and nitrate show frontal excursion past the fixed station (Fig. 5). Surface water displacement produced a large gradient both in chlorophyll and in nitrate concentrations near the surface; however, nitrate concentrations remained relatively constant below about 40 m. The photic zone depth (depth where light intensity is 1% of the surface value) was 40 m at station 2010 outside the chlorophyll maximum region, 14 m at station 2016,

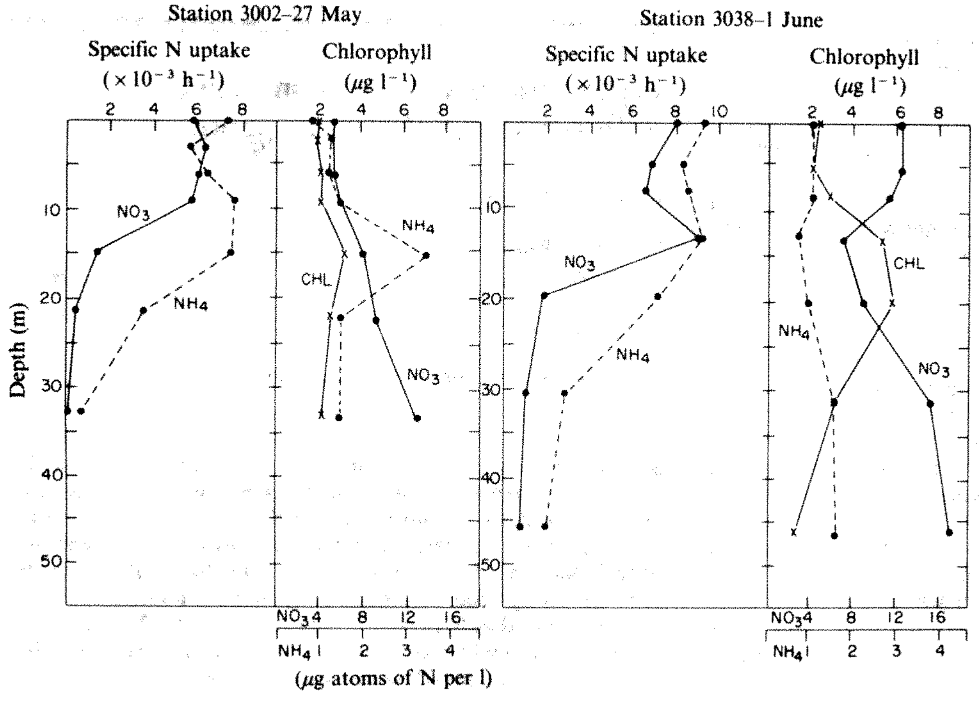

Fig. 4 Nitrate, ammonium, and chlorophyll concentrations measured from hydrocast water samples. Specific nitrogen uptake velocities for phytoplankton from the hydrocast samples held 24 hours in a deck incubator at the light intensity at the depth where they were collected.

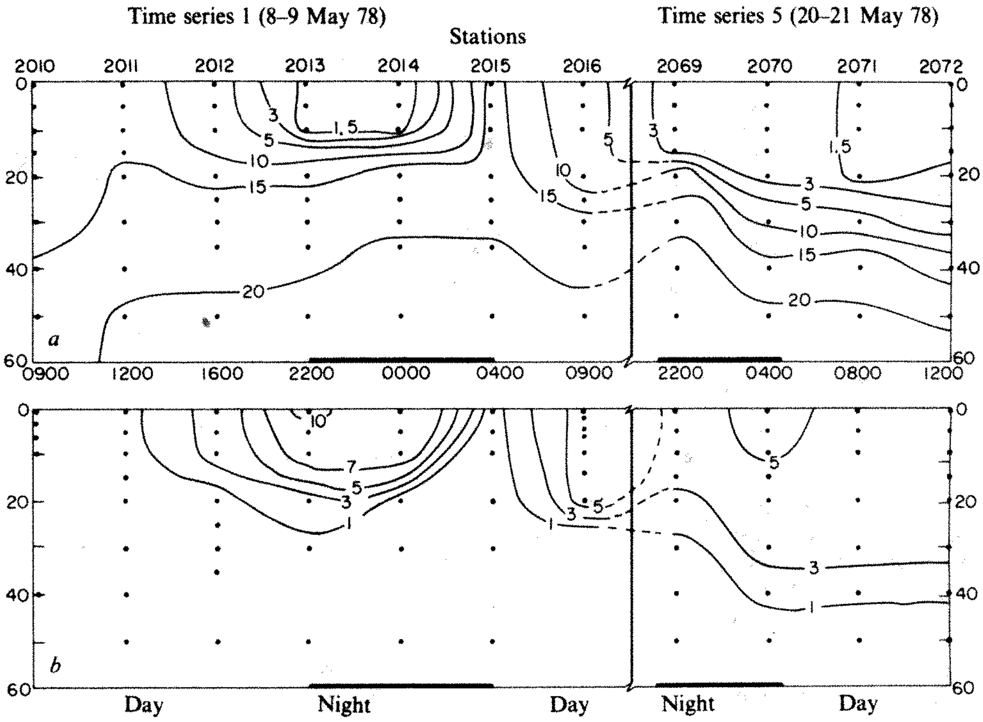

Fig. 5 *a*, Nitrate concentration (μg atoms of NO_3 per 1 of N) and *b*, chlorophyll concentration (μg chl per 1) measured at the time series station.

and 16 m at station 2071 near the chlorophyll maximum. Productivity below 20 m at station 2071 was zero for ^{14}C estimates. An indication of the persistence of chlorophyll and nitrate patterns in the front with time can be seen in the continuation of the patterns in chlorophyll and nitrate concentrations with depth at the same station two weeks later. The station was located with Loran C, considered accurate to within 200 m in the southeastern Bering Sea. A significant portion of the water column chlorophyll had settled to the base of the photic zone by the end of the second phase of the time series, although vertically integrated chlorophyll values were similar at stations 2013 and 2071. Silicate was nearly constant between 28 and 30 μg atoms of Si per 1 in the photic zone during the time series, supporting a contention that diatoms were not a major constituent of the phytoplankton in the shelf break front.

Nitrogen uptake velocities obtained with ^{15}N nitrate and ammonium allow estimation of nitrogen doubling times for phytoplankton communities. A doubling time of about 4 days was calculated for *Pheocystis poucheti* which numerically dominated the phytoplankton at station 3002 (Fig. 4). The ammonium maximum at 15 m was coincident with a shift in phytoplankton species composition to a community co-dominated by *Biddulphia* sp. and *Prorocentrum* sp. which doubled in about 3.5 days. A doubling time of about 5 days was calculated for the surface sample at station 3038 where *Cylindropyxis tremulens* and *Thalassiosira aestivalis* dominated the phytoplankton. Nitrogen doubling times were similar up to 15 m, below which doubling times ranged from 16 to 30 days. Nitrogen uptake was observed at the 0.1% light depth; however, carbon fixation was usually not different from zero at the 1% light depth. There was a transition from *Pheocystis poucheti* which formed blooms near the surface to a phytoplankton community dominated by diatoms and dinoflagellates as the photic zone deepened with the sinking of the chlorophyll maximum layer in the shelf break front.

The pattern exhibited by chlorophyll and nitrate maps and in hydrocast data suggests that the phytoplankton bloom in the shelf break front of the southeastern Bering Sea was constrained to a narrow ribbon parallel to the 100 fathom isobath. A chlorophyll maximum was always observed along the 170 km length of the front but the maximum was not always at the surface. The chlorophyll maximum persisted in the shelf break front through September 1978 and was observed again during spring 1979.

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Hypersaline solutions interact with organic detritus to produce oil

Egon T. Degens

Geologisch-Paläontologisches Institut, Universität Hamburg, Hamburg, FRG

Antonin Paluska

Geologisches Landesamt Hamburg, Hamburg, FRG

Salt is a major factor in the origin of petroleum. It leads to the extraction of hydrocarbons from sediments and the formation of oil droplets. Also, salt domes and their cap rocks may trap oil[1]. Although the diagenesis of salt and brines is well understood, little is known what salty brines may do to infalling organic detritus. Using results from the Caspian Sea and the North Sea we show here that hypersaline solutions close to the sediment-water interface will cure organic matter and that this 'pickling effect' is essential for obtaining black and oil shales.

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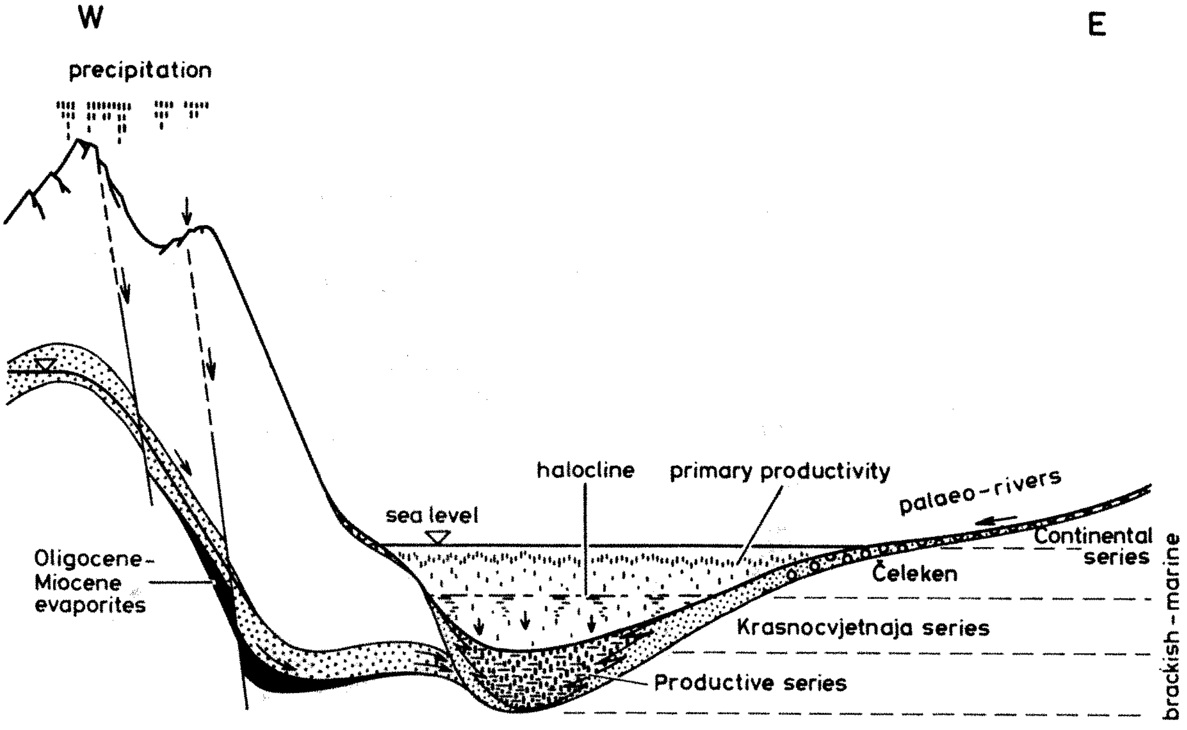

Fig. 1 South-Caspian depression during Kimmer/Akchagyl time; evaporite series are shown in black.

The Black Sea is considered the prototype environment for the black shale facies[2]. During the RV Glomar Challenger DSDP Leg 42B a series of black shales, oil shales and sapropels of Quaternary age were recovered from the Black Sea deep basin at a water depth of about 2,000 m (ref. 3). The 'euxinic events' responsible for this facies pattern are all characterised by saline spills or brine invasions, which in turn led to a midwater halocline and a meromictic basin. Note that oil shale facies develops in a shallow water environment at a water depth of at most a few hundred metres[4]; hypersaline conditions with salinities reaching 100‰ are indicated[5]. In contrast, sapropels are confined to a deep water meromictic sea and salty brines covering the topmost sediment are of lower salinity[4]. Oil shales have ages between 1 and 2 Myr, an organic C content between 0.5 and 2%, and downhole temperature measurements indicate a thermal gradient between 30 and 50 °C per km (ref. 6). These results suggest that hydrocarbon formation in the Black Sea was fast, proceeded at low temperature, and was mediated by syngenetic salt invasions.

In the light of these data we examined two test regions, the Caspian Sea and the North Sea, where we believe similar environmental conditions did prevail except that the geological processes have already led to the accumulation of sizeable oil deposits[7].

During the Pontic marine phase (lower Pliocene) wide parts of Russia and southeastern Europe were covered by a land-locked shallow sea[8]. The subsequent Kimmer regression reduced the sea to a few isolated basins located in the region of the present south-Caspian depression[9]. The Kimmer climate was arid and the palaeo-rivers Volga, Amudarja and others carried a high salt load to a comparatively small Caspian drainage basin. Pliocene orogeny, still active during the Kimmer, led to the formation of mountains in the vicinity of the Caspian Sea[10]. Groundwater under high pressure within the tilted rock formation moved towards the south-Caspian depression where it was released in the form of Artesian wells. Since the aquifer drained evaporites of Oligocene–Miocene age, mineralisation of the groundwater must have been substantial.

The geological and environmental situation around Kimmer and Akchagyl time is depicted in Fig. 1. Bleeding of groundwater aquifers below sea level combined with brine migration from lagoons resulted in the accumulation of salty brine pools in morphological deeps; a midwater halocline separated the brines from normal Caspian Sea water. The Akchagyl transgression following the Kimmer regression produced a facies succession from clays to sands to gravel (Productive, Krasnocvjetn Čeleken, Continental series); brackish-marine and continental deposits mark this event[11]. High primary productivity is indicated, and consequently the Productive series below the halocline received a high input of organic matter and the anoxic conditions established preserved the infalling organic detritus. As the Akchagyl transgression took place at about the Tertiary/Quaternary boundary, generation of hydrocarbons and formation of oil traps must have been quite rapid.

Our second test case, the North Sea, follows an almost identical pattern, except that the black shale event was much earlier, during the Upper Jurassic[12]. A schematic cross-section along a line from Norway to Scotland at the time of the formation of the Kimmeridge clay (Malm) depicts the functionality of the Zechstein and Keuper evaporite series for the generation of a salty groundwater aquifer (Fig. 2). Such a mineralisation and the subsequent release to the basin of deposition was possible at Kimmeridge time because the potential needed for the Artesian pressure was generated at that time by tectonic uplift in Scandinavia and tectonic subsidence in the vicinity of the Central Graben. The water depth in the Central Graben during the Upper Jurassic has been estimated to be at about 1,000 m and a few hundred metres close to the shelf area[13]. In short, the generation of oil shale facies required euxinic conditions and a hypersaline syngenetic environment close to the sediment–water interface. Note that the generation of hydrocarbons could already have started during the Kimmeridgian tectonic phase. The presence of North Sea oil in younger rock formations, for instance, of the early Tertiary, may be linked with hypersaline anoxic events in the Eocene and Oligocene. Alternatively, a

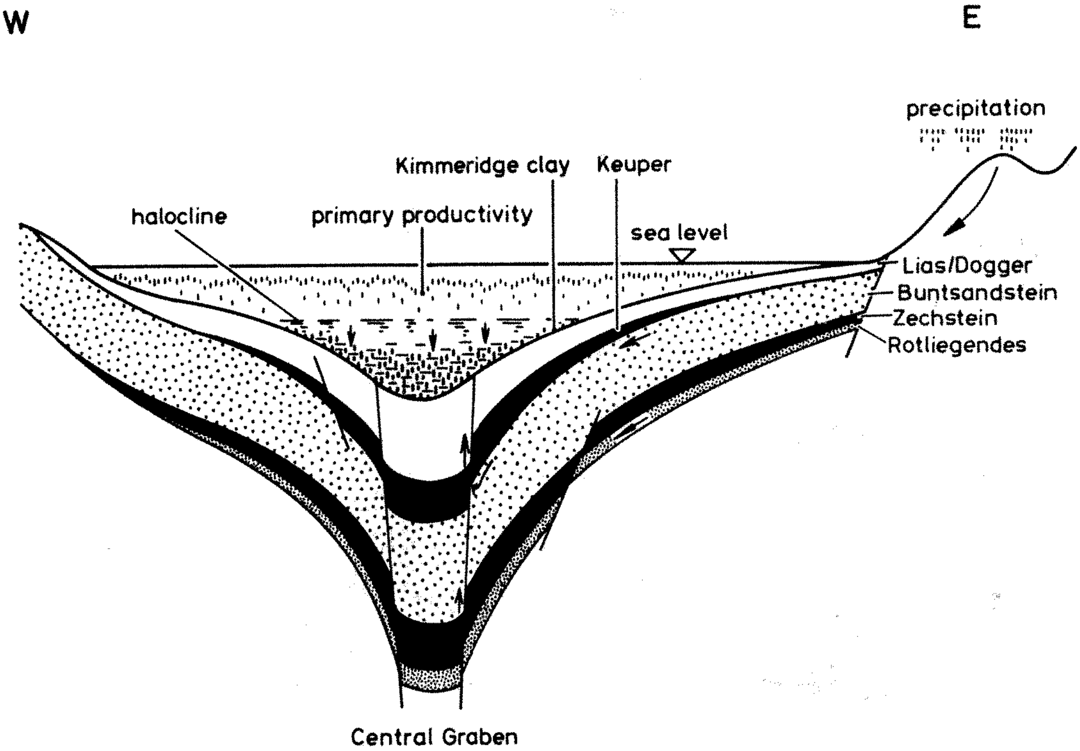

Fig. 2 North Sea at Kimmeridge time; evaporite series are shown in black.

migration of Jurassic petroleum into younger reservoir rocks is conceivable.

Salt water driven by Artesian forces is presently expelled from Red Sea sediments and accumulates in the so-called 'hot brine pools' (ref. 14). The brines are released in pulses which are in phase with pluvial incidents. An identical situation exists in some deep lakes of the East African rift system, most notably in Lake Kivu[15]. In both areas the brines extend from the sea or lake floor up to 200 m into the open water column and are separated from the upper water layer by one or a series of haloclines. Such a decoupling of the upper from the lower water layer will generate anoxic conditions at depth.

Salt invasions will drastically affect marine life in sediment and water. Higher forms of aquatic life become wiped out completely, and microbial population patterns undergo distinct changes in response to, for example, the degree of salinity, ionic composition or brine temperature[16]. Some of these changes are limiting, others are accelerating factors for microbial productivity. High general salinity combined with high calcium content might even act bacteriostatically on sulphate-reducing bacteria[17]. In contrast, halophilic bacteria may thrive in salty waters[18]. The establishment of characteristic microbial food chains or food webs adapted to the new environmental conditions can lead to a transformation of low molecular weight carbon compounds such as CO, CO_2, CH_4, organic acids into bacterial biomass[18]. The microbial complexity of brine systems is well documented in the work on Red Sea brine pools[17], Lake Kivu[15] or the Black Sea[19].

With respect to oil shale formation the most favourable environment seems to be one in which decomposition of high molecular weight organic matter is kept low and synthesis of bacterial biomass from low molecular weight carbon compounds is high. In such conditions, infalling organic debris of marine and terrestrial origin has a good chance of 'surviving' early diagenesis. This, combined with additional increments from microbiological organic matter, yields the high molecular weight precursor material for the generation of oil.

The formation of oil shales requires high primary productivity, hypersaline conditions at depth, and a gradually subsiding basin. Microorganisms such as methane producers, methane oxidisers (close to the halocline), halophilic bacteria or thermophilic bacteria will supplement the organic input into the sediment. The activity of organisms consuming organic matter, such as sulphate reducers, must be low. The salty waters will sink into the sediment and impregnate the rock formation. Most of the salt in petroleum brine waters has this point of origin. Curing by salt will preserve and condense organic matter. For this reason kerogen in oil shale is of a different nature from kerogen in an ordinary marine or freshwater shale. In taking these environmental and geological factors into consideration, oil exploration may take on some new dimensions.

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Oldest known eutherian stapes and a marsupial petrosal bone from the Late Cretaceous of North America

J. David Archibald

Department of Biology and Peabody Museum of Natural History, Yale University, New Haven, Connecticut 06520

Cranial remains of Late Cretaceous therian mammals are rarely found in North America and are usually limited to isolated petrosal bones. The oldest known associated stapes and petrosal bone of a Late Cretaceous eutherian are reported here. Comparison with a Late Cretaceous marsupial petrosal bone gives a further indication of the probable condition of several character states of the ancestral mammalian (or at least therian) middle ear, and also indicates the antiquity of various aspects of the middle ear of extant eutherians and marsupials.

A worn and isolated eutherian petrosal, lacking most of the mastoid process, was identified in the collections of the US National Museum of Natural History. The specimen, USNM 215118 (Fig. 1*a*, *e*), comes from the Late Cretaceous Bug Creek Anthills local fauna (Hell Creek Formation, McCone County, Montana)[1]. MacIntyre[2] described several well preserved eutherian petrosals from this local fauna which he called 'ferungulate' and 'unguiculate' types. The former probably belongs to the arctocyonid condylarth, *Protungulatum donnae*, and the latter to the palaeoryctid proteutherian, *Procerberus formicarum*. USNM 215118 is of the latter type and is unique in preserving a broken but complete stapes wedged in the cochlear labyrinth (Fig. 1*a*, *b*). The other petrosal described here also comes from the Hell Creek Formation, but about 60 km to the west, in Garfield County, Montana. This well preserved specimen, UCMP 118262, is almost certainly a marsupial, based on its provenience and morphology. It is slightly smaller than the only other known Late Cretaceous marsupial petrosal, that of the stagodontid, *Didelphodon vorax*[3]. Based on size, UCMP 118262 probably belongs either to one of the pediomyids, *Pediomys hatcheri* or *P. florencae*, or to the didelphid, *Alphadon rhaister*.

The stapes associated with the eutherian petrosal has an elongate footplate. The crura are quite gracile and set near either end of the footplate, and the stapedial foramen is large. The stapedial muscle probably inserted on the capitulum or on the thickened portion of the posterior crus immediately below (medial to) the capitulum (is, Fig. 1*b*). This stapedial morphology is seen in various extant eutherians[4]. Although the Late Cretaceous marsupial petrosal has no stapes, the shape of the footplate can be estimated from the shape of the fenestra vestibuli (fv, Fig. 1*f*), as can be done for the Late Cretaceous eutherian petrosal (fv, Fig. 1*a*) because in mammals the fenestra vestibuli outlines the shape of the adjacent stapedial footplate.

Segall[5] quantified the shape of the footplate or fenestra vestibuli as the ratio of length to width. Among didelphid marsupials he found ratios of 1.3–1.5, with the exception of *Dromiciops* at about 2.1. *Dromiciops* has a highly modified ear region and is not considered to be a didelphid by some authors[6]. The fenestra vestubuli of UCMP 118262 has a ratio of about 1.4, the same as for *Didelphodon*. This value lies within the range measured for modern didelphids. In contrast, the eutherians (mostly lipotyphlans) examined by Segall have ratios ranging from 1.8 to 2.9. The ratio in the new eutherian petrosal, USNM 215118, is 2.4 based on the stapedial footplate and 2.1 based on the slightly damaged fenestra vestibuli. The figures of the 'ferungulate' eutherian petrosal of MacIntyre[2] also indicate a ratio of 2.0 or more based on the fenestra vestibuli.

Primitvely, mammals (and advanced mammal-like reptiles[7]) had a circular or possibly slightly oval stapedial footplate (equal to a ratio of about 1.0). Thus, the elongate footplates of the new Late Cretaceous eutherian, many extant eutherians and some extant marsupials (such as *Macropus*[5]) represent a derived condition. The smaller ratio in extant didelphid marsupials and in the new Late Cretaceous marsupial petrosal indicates a less elongate stapedial footplate, resembling more closely the circular footplate primitive for mammals.

There is less agreement about the primitive mammalian condition of the crura of the stapes. In monotremes and some marsupials with a circular or slightly oval footplate, the stapes is

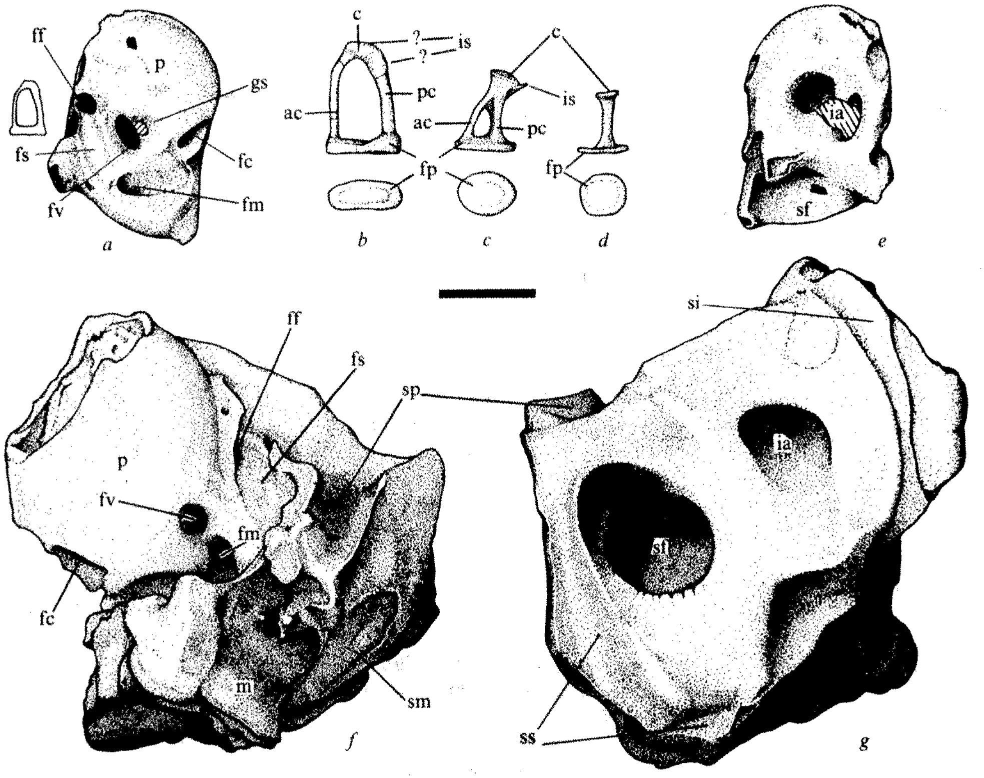

Fig. 1 Right petrosal (*a*, *e*) and stapes (*a*, *b*) of 'unguiculate' eutherian, USNM 215118, and left petrosal (*f*, *g*) of marsupial, UCMP 118262, both from Hell Creek Formation, Montana; right stapes of *Didelphis virginiana* (*c*) and *Tachyglossus aculeatus* (*d*); *a*, *f*, ventrolateral view, anterior approximately towards top; *b*, *c*, *d*, dorsal and medial views, anterior to left; *e*, *g*, dorsomedial view, anterior approximately towards top. ac, anterior crus; c, capitulum; fc, fenestra cochleae; ff, foramen for facial nerve; fm, fossa for origin of stapedius muscle; fp, footplate; fs, sulcus for facial nerve (broken bony canal in marsupial); fv, fenestra vestibuli; gs, groove for stapedial artery; ia, internal acoustic meatus; is, insertion for stapedius muscle; m, mastoid portion of petrosal; p, promontorium of petrosal; pc, posterior crus; sf, subarcuate fossa (broken in eutherian); si, sulcus for inferior petrosal sinus; sm, sulcus for vein leading to mastoid foramen; sp,? sulcus for portion of transverse sinus leading to postglenoid foramen; ss, posterodorsal portion of sulcus for sigmoid sinus. Scale bar, 2 mm (*a*, *d*, *e*, *f*, *g*) and 1 mm (*b*, *c*).

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columelliform and lacks a stapedial foramen. Segall[5] believes that this is the primitive mammalian condition. Henson[8] follows Goodrich[9] in believing that it is a derived condition.

In addition to many extant mammals, some extant lizards, birds and amphibians[8,9] have a perforate stapes (possess a foramen) that transmits the stapedial artery. In some mammal-like reptiles such as *Thrinaxodon*[7] the stapes had a large stapedial foramen. Whether a stapedial artery traversed this foramen is not known, but it seems likely. The perforate stapes of most mammals and various other fossil and extant tetrapods suggests that this was the primitive mammalian, if not primitive tetrapod condition. Thus, the primitive condition of the crura of the stapes among mammals would resemble the morphology of *Didelphis* (Fig. 1*c*) more closely than that of a monotreme (Fig. 1*d*). Both the columelliform crus of the monotreme stapes and probably the stirrup-like crura of the Late Cretaceous eutherian stapes (Fig. 1*b*) would be derived conditions.

The two new petrosals show several grooves for parts of the arterial and venous systems. Such grooves indicate the patterns of these systems in later fossil and extant eutherians and marsupials. Due to wear, the Late Cretaceous eutherian petrosal has only the shallow, but distinct groove for the stapedial artery (gs, Fig. 1*a*). Based on better specimens of the same type of petrosal[2], it almost certainly also had grooves for the medial internal carotid and promontory arteries. In contrast, the well preserved Late Cretaceous marsupial petrosal shows no trace of grooves for any of these arteries.

Both patterns are consistent with what is known of the evolution of the internal carotid circulation in eutherians and marsupials. The common ancestor of eutherians and marsupials had a medial internal carotid that passed medial to the petrosal, leaving little or no trace on this bone, and a stapedial artery that pierced the stapes, possibly leaving some trace on the petrosal[10]. Various lines of evidence suggest that the earliest eutherians maintained this general pattern, adding a neomorphic vessel, the promontory artery, which crossed the lateral side of the promontorium[10]. However, embryological evidence[11] suggests that the eutherian promontory artery represents a shift of the medial internal carotid to the promontorium.

In contrast, the evidence from extant marsupials indicates they neither developed a promontory artery nor shifted an artery to this position[10]. In addition, reports of a branch of the internal carotid passing through a groove or bony canal on the medial side of the marsupial petrosal[12,13] have not been substantiated[14]. Thus, such a groove in the Late Cretaceous marsupial petrosal (si, Fig. 1*g*) carried a portion of the venous system, the inferior petrosal sinus, not a branch of the internal carotid. Extant marsupials that have been studied usually have the medial internal carotid, while the stapedial artery is reduced[15]; seldom does either leave a trace on the petrosal. *Didelphodon* and the new Late Cretaceous marsupial petrosal similarly lack grooves for the medial internal carotid and stapedial arteries. By analogy, if not homology, both Late Cretaceous marsupials probably had a medial internal carotid, but lacked or had a greatly reduced stapedial artery.

These comparisons indicate the antiquity of several characters of the middle ear seen in various extant eutherians and marsupials. By the Late Cretaceous the stapes had been modified in at least some eutherians. Functional implications remain speculative. At the simplest mechanical level, the torque about the long axis of an elongate stapedial footplate such as in the Late Cretaceous stapes is less than about the axis of a circular footplate of the same area. Thus, even such a minor modification as the lengthening of the footplate may have enhanced the hearing capabilities of this eutherian. The much less elongate footplate (indicated by the shape of the fenestra vestibuli) of Late Cretaceous marsupials is a retention of a more primitive condition that is maintained in extant didelphids. The elongate footplate in various other extant marsupials evolved in parallel with the eutherians.

In contrast to stapedial morphology, the portions of the internal carotid system that cross the middle ear show a pattern of mosaic evolution in Late Cretaceous eutherians and marsupials. The common ancestor of mammals almost certainly possessed a medial internal carotid and a stapedial artery. By the Late Cretaceous, marsupials had greatly reduced or lost the latter vessel, whereas contemporaneous eutherians maintained it. Also, if the interpretations of the various grooves are correct, eutherians had developed a neomorphic vessel, the promontory artery, by the Late Cretaceous. Again, functional implications are speculative, but these various patterns may simply reflect a shifting of the arterial blood supply to portions of the brain and orbit; or in the case of eutherians, the probable appearance of a new artery may have served as a sort of primitive rete mirabile for cooling blood destined for the brain.

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Effective mutualism between sequentially flowering plant species

Nickolas M. Waser

Department of Biology, University of California, Riverside, California 92521

Leslie A. Real

Department of Zoology, North Carolina State University, Raleigh, North Carolina 27650

Since the theoretical paper of Levin and Anderson[1], it has been widely recognised that animal pollinators represent resources for which plants can compete. Competition for pollination might take several forms and act as a powerful selective force in establishing or maintaining sequential flowering among sympatric species[2]. Sequential flowering in Arctic[3], temperate[4–9] and neotropical[10–13] plant assemblages has been interpreted as an evolutionary result of competition for pollination. This interpretation may prove correct in many cases, although strong evidence for competition is available for few systems[9,14]. Heinrich and Raven[15] (see also Baker[16] and Baker *et al.*[17]) pointed out that sympatric plant species may act as mutualistic partners at the same time that their sequential flowering is maintained by competition. We develop this hypothesis here explicitly and present evidence that effective mutualism occurs between two species which also compete for pollination.

To illustrate the hypothesis of mutualism we consider three sequentially flowering species which share pollinators (Fig. 1). If

Table 1 Densities of flowers, seed sets and census counts of hummingbirds from the RMBL

Year	Peak density of *D. nelsonii* flowers*	Peak density of *I. aggregata* flowers*	*I. aggregata* seed set†	Overall broad-tail census count‡	Late broad-tail census count§	Overall rufous census count‖
1975	25.0±9.20 (6)	29.3±13.26 (6)	10.1±0.73 (37)	10.2±1.44 (14)	8.6±2.09 (7)	7.1±2.79 (8)
1976	30.8±9.41 (6)	27.8±9.32 (6)	12.4±0.68 (47)	—	—	—
1977	0.8±0.54 (6)	18.7±7.70 (6)	7.6±0.46 (59)	8.0±1.13 (11)	6.9±1.45 (7)	7.2±6.95 (5)
1978	7.2±2.36 (6)	39.3±13.99 (6)	7.1±0.71 (43)	5.0±1.86 (10)	5.0±1.86 (10)	6.9±2.43 (8)

* Values are mean number of flowers per 4 m^2 plot ±1 s.e.m. with sample sizes (no. of plots) in parentheses. Both species were counted every 2–3 days in 6 permanent census plots. The 1977 *D. nelsonii* value is a slight underestimate as the first census was taken when density of this species was already declining.

† Values are mean seed sets per flower ±1 s.e.m., with sample sizes (no. of flowers) in parentheses. Flowers were taken from unmanipulated plants in the meadow containing flower census plots.

‡ Values are mean number of flights by male broad-tailed hummingbirds per 10 min census ±1 s.e.m., with sample sizes (no. of censuses) in parentheses. Flights were censused at a standard time of day in the meadow containing flower census plots; no censuses were conducted in 1976. Censuses cover the flowering season of both species in 1975 and 1977 and flowering of *I. aggregata* alone in 1978.

§ Values are given as before. Only censuses conducted during flowering of *I. aggregata* have been included for each year.

‖ Values are given as before, except for male rufous rather than broad-tailed hummingbirds. Censuses cover the flowering season of *I. aggregata* only, as rufous hummingbirds were not present during flowering of *D. nelsonii*.

the time between peaks of flowering of species 1 and 3 is sufficient for the absence of species 2 to cause appreciable mortality, emigration, or loss of fecundity to pollinators, then species 2 increases immediate reproductive potential of species 3 by maintaining pollinators through some time interval t. When the flowering season (the period $T_1 \rightarrow T_2$) covers an entire year all three species are engaged in mutualism. When $T_1 \rightarrow T_2$ represents part of a year, a similar interaction occurs if colonisation, survival, or fecundity of pollinators depend on overall abundance of flowers or on their continuous availability throughout some critical period.

For this mutualism to occur numbers of pollinators must be reduced locally in response to reduced numbers of flowers. This seems especially likely for obligate flower visitors such as bees, bombyllid and syrphid flies, hawkmoths and most butterflies[18–20]. For example, survival and reproduction of bumblebee colonies seem to reflect availability of flowers[21]. Pollinators which are not obligate flower visitors also may react strongly to reduced flowering. For example, densities of territorial hummingbirds and sunbirds reflect densities of flowers[22–25] and poor flowering is associated with reduced survival and nesting success of hummingbirds[25–27].

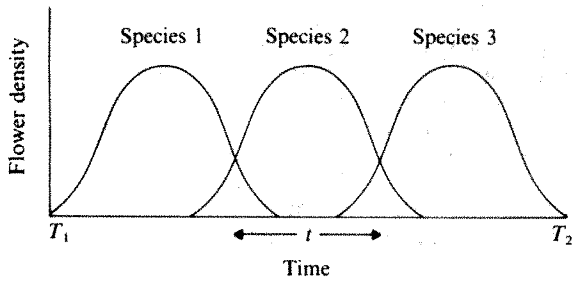

Fig. 1 Idealised sequential flowering of three sympatric species sharing common pollinators. Removal of any species leaves a period t during which few or no flowers are available locally.

Our previous work[9] suggests that competition for hummingbird pollination maintains sequential flowering of two herbaceous wildflowers, *Delphinium nelsonii* Greene and *Ipomopsis aggregata* (Pursh) V. Grant, around the Rocky Mountain Biological Laboratory (RMBL) in Colorado. From measurements of seed sets and densities of flowers over four summers we have evidence that these two species are also engaged in a mutualism of the kind we describe. Peak densities of *D. nelsonii* flowers were similar in 1975 and 1976 but declined drastically in 1977 and 1978 (Fig. 2, Table 1), presumably due to the 1977 winter drought. Densities of *I. aggregata* flowers remained relatively constant between years and were uncorrelated with densities of *D. nelsonii* flowers (Table 2*a*). The mutalism hypothesis predicts that reproductive success of *I. aggregata* will decline in the 2 years of low density of *D. nelsonii*. This prediction is supported by reduced seed sets of *I. aggregata* in 1977 and 1978 (Table 1). The slope of the linear regression of seed sets on peak densities of *D. nelsonii* flowers over all years is positive, and the correlation between these variables is significant (Table 2*b*).

It could be argued that reduced fecundity of *I. aggregata* in 1977 and 1978 was also a subtle effect of drought rather than of reduced availability of hummingbird pollinators after failure of *D. nelsonii* flowering. However, census records suggest that activity of male broad-tailed hummingbirds (*Selasphorus platycercus* Swainson) was reduced in 1977 and 1978 relative to 1975 as predicted by the mutualism hypothesis. These birds, along with rufous hummingbirds (*S. rufus* Gmelin), are the major pollinators of *I. aggregata* around the RMBL[9], and this species is self-incompatible (1978 seed sets of self-pollinated flowers on five bagged plants compared with those of open-pollinated flowers on eight unbagged plants were 0.3±0.08 (n = 256) compared with 7.1±0.71 (n = 43). However, rufous hummingbirds are migratory transients who usually arrive after flowering of *D. nelsonii* and whose activity did not fluctuate substantially

Table 2 Linear regressions and correlations between seed sets, densities of flowers and census counts of hummingbirds

a Peak densities of *I. aggregata* flowers against peak densities of *D. nelsonii* flowers

I. aggregata = 0.08 (*D. nelsonii*) + 27.44
$r = 0.14$ d.f. = 2 $P > 0.5$ NS

b Raw *I. aggregata* seed sets against peak densities of *D. nelsonii* flowers

seed set = 0.15 (*D. nelsonii*) + 7.04
$r = 0.41$ d.f. = 183 $P < 0.001$

c Raw *I. aggregata* seed sets against overall census counts of broad-tailed hummingbirds

seed set = 0.58 (census count) + 3.65
$r = 0.27$ d.f = 136 $P < 0.01$

d Raw *I. aggregata* seed sets against late census counts of broad-tailed hummingbirds

seed set = 0.78 (census count) + 2.90
$r = 0.24$ d.f. = 136 $P < 0.01$

All regressions were run using mean values from Table 1, except that *I. aggregata* seed sets are original raw values rather than means for each year. Correlation coefficients and significance values are shown for the association between each set of variables. NS, not significant.

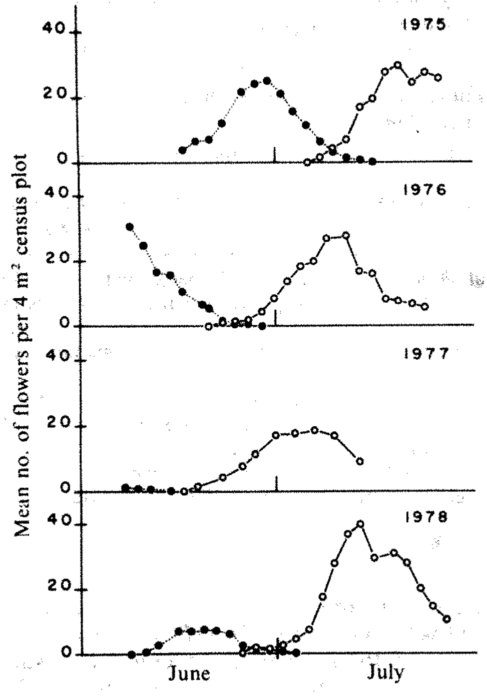

Fig. 2 Sequential flowering of *D. nelsonii* (●) and *I. aggregata* (○) at the RMBL during four consecutive summers. Note the poor flowering of *D. nelsonii* in 1977 and 1978.

between years (Table 1). In contrast, broad-tailed hummingbirds are breeding residents who colonise the RMBL area largely in response to flowering of *D. nelsonii*[27]. Slopes of linear regressions of *I. aggregata* seed sets on yearly mean census counts of broad-tailed hummingbirds are positive, and correlations between these variables are significant (Table 2*c*, *d*).

In contrast to competition for pollination, it is difficult to see how the mutualism discussed here could establish or maintain sequential flowering through selection acting on individual plants or even on alternate plant assemblages. We consider effective mutualism to be an unanticipated outcome, rather than a cause, of divergence in flowering times of sympatric species.

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'Time–intensity trading' in locust auditory interneurones

Jürgen Rheinlaender & Alfred Mörchen

Lehrstuhl für Allgemeine Zoologie, Ruhr-Universität Bochum,
Postfach 102148, 4630 Bochum, FRG

In the locust the firing rate of tympanal nerve fibres encodes the direction of the sound source, and this has been used as fundamental evidence for the directional sensitivity of each of the tympanal organs[1–4]. As there is a minute disparity between the two organs (in *Locusta* this distance is about 4 mm), it was generally accepted that the physical time cue—maximally about 10 µs—cannot be used for sound localisation. However, Mörchen *et al.* have found that in single receptor fibres of the locust, the magnitude of excitation is highly correlated with the latency of nervous activity[5]. In their study a sound source was moved around the animal and the latency of a neural discharge increased by up to 6 ms as the firing rate decreased. This 'neural time cue' exceeded the physical one by a factor of 100–1,000. Comparison of the inputs from the two organs shows that there are two neural codes available (spike count and latency), each of which can adequately specify the direction of the sound source. We report here that the subsequent synapses process this dual mode of directional coding and that both the rate of binaural spike discharges and latency differences influence the activity of auditory interneurones.

We recorded the responses of a pair of identified acoustic interneurones in *Locusta migratoria* from the connectives between the meso- and metathoracic ganglia. This pair of auditory units (type B_1)[6] is excited ipsilaterally and inhibited from the contralateral side, and thus it is appropriate for the study of binaural processing. To provide an effective uncoupling of the two organs the tympanal membranes were displaced by contact-mechanical stimulation. Two custom-made piezoelectric transducers (similar to those described elsewhere[7]) were used and driven with a sinusoidal signal of 69 kHz; such a frequency eliminates conventional acoustic stimulation of auditory receptor cells (compare ref. 8) but nevertheless activates them adequately (for control experiments see below). With this method the 'crosstalk barrier' between both ears was greater than 55 dB. In each experiment the activities from the units on both sides of the central nervous system (CNS) were recorded successively to calibrate the stimulus intensities of the two probes (for control experiments see below).

The effects of interaural time and intensity differences on the response of the auditory interneurone are shown in Fig. 1. The solid line represents equal stimulus intensities at both ears. By stimulating the ipsilateral (excitatory) ear alone, a maximal response was achieved; this corresponded to the binaural stimulus situation with the ipsilateral stimulus preceding the contralateral (inhibitory) stimulus in a range from 4 to 40 ms. When the inhibitory stimulus preceded the excitatory one the activation of this unit was completely suppressed (note that the steep slope of the curve occurred in a time window of ~ ±2 ms for either the ipsi- or contralateral stimulus leading). Thus the synaptic inputs of this interneurone are highly sensitive to time differences of the order of a few milliseconds. It was necessary for the inhibitory stimulus to precede the excitatory one by more than 80 ms for it to be completely ineffective (not shown in Fig. 1, for comparison see Fig. 2).

A decrease in the contralateral (inhibitory) stimulus of only 5 dB shifted the steep slope of the curve in Fig. 1 to the left (dotted line) where the midpoint is defined by simultaneous stimuli. This means that inhibitory effects were only effective when preceding excitation by more than 2 ms, but not suppressing the activity completely. When the contralateral stimulus was reduced by a further 5 dB, it had no effect on the response of the interneurone. However, when the intensity of the contralateral

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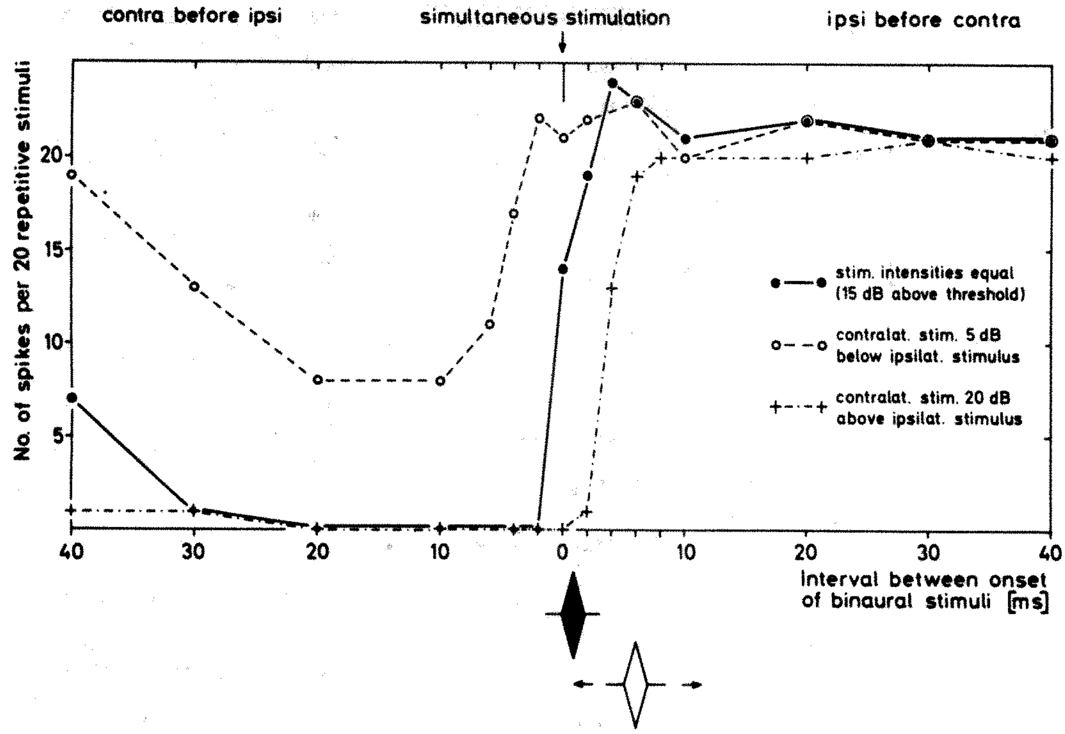

Fig. 1 Effects of interaural time and intensity differences on the response of an auditory interneurone in the locust to short contact-mechanical stimuli on the typanic membranes; activation by ipsilateral (◆), inhibition by contralateral (◇) stimulus (stimulus frequency 69 kHz, rise- and decay-time 1 ms, plateau 0 ms, repetition rate 1 per s), for further indications see other figure legends. Note that the steepest slope of the curves produced by various interaural time differences can be traded by changing the interaural intensities.

stimulus was increased relative to the ipsilateral one the steep slope of the curve shifted to the right (shown in Fig. 1 for 20 dB difference, dashed line). This means that with more intense contralateral stimuli the inhibition was effective even when it followed the ipsilateral stimulus by ~4 ms. (A further increase of the contralateral stimulus intensity had no additional effect on this curve.)

The data presented in Fig. 1 reveal that in the CNS of insects there is a phenomenon similar to the time–intensity trading well known for vertebrates (reviewed by Erulkar[9]). But in insects the 'time cue' is based on the physiological properties of scolopidian receptor cells and has another order of magnitude. Furthermore, the data show that within a given 'time and intensity window' such interneurones are highly sensitive to binaural changes. Specifically interaural time differences of the order of 4 to 6 ms and interaural intensity differences of about 20 dB will change the activity from maximal excitation to total inhibition.

The experiments with contact-mechanical vibration may present some technical problems (for more details see ref. 10). In particular, with bilateral processing, the question of whether there is symmetrical displacement of the two membranes, and the equivalence of acoustic and contact-mechanical stimulations have to be tested. To answer the first question a control measurement was performed in each experiment and the activities from equivalent units on each side of the CNS were recorded in succession. This procedure was necessary to define the neural thresholds of each unit to the vibratory stimulus, providing controlled stimulus intensities at both ears. In some cases (such

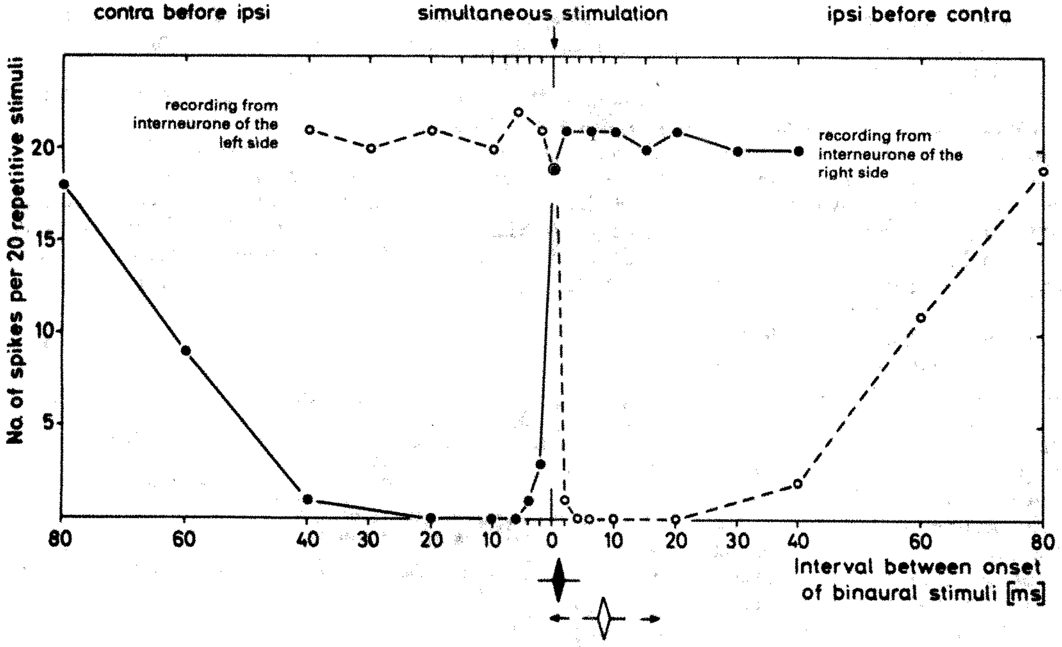

Fig. 2 Effects of interaural time difference on the response of a pair of auditory interneurones on either side of the CNS in the locust to short contact-mechanical stimuli on the tympanic membranes; activation by ipsilateral (◆), inhibition by contralateral (◇) stimulus, 15 dB above threshold respectively (further stimulus configurations see Fig. 1 legend). In each recording the 'leading' trigger and recording side have been reversed. Note the symmetry and the steepest slope of the curves near simultaneous stimulus onsets.

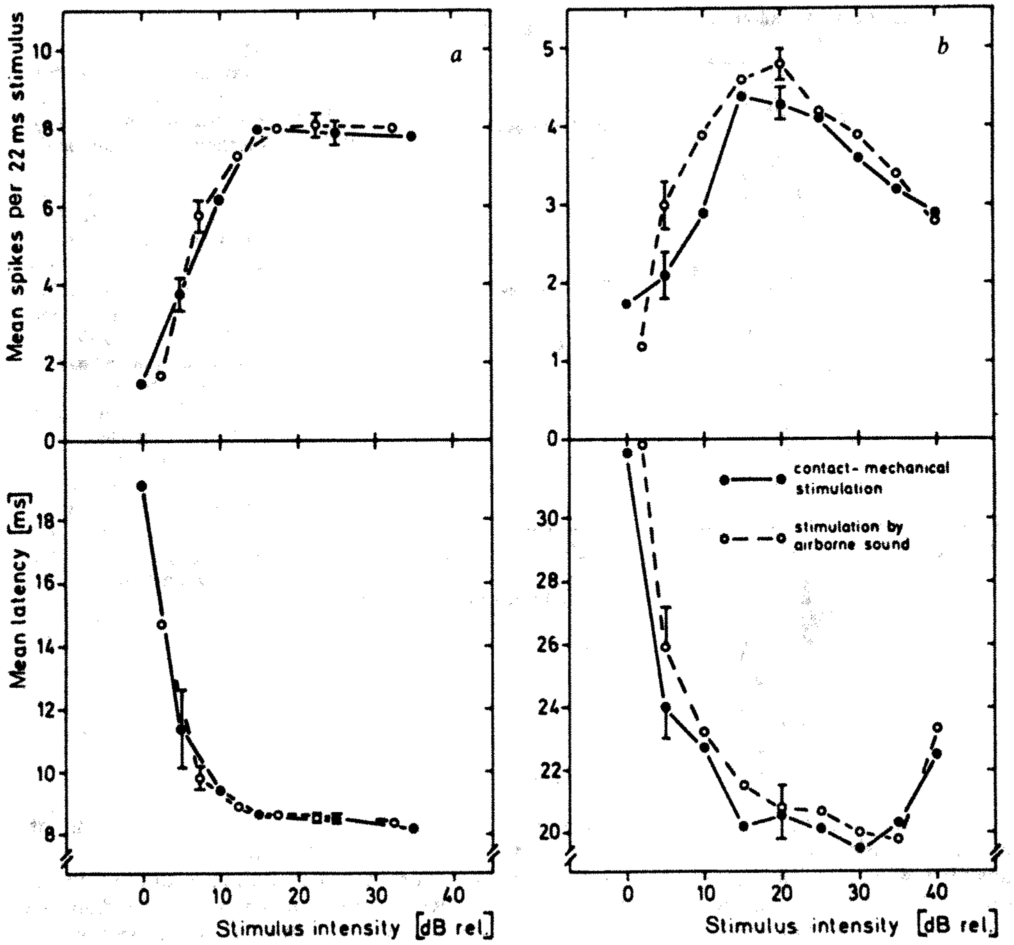

Fig. 3 Intensity and latency characteristics of a receptor cell (*a*) and an interneurone (*b*) of the locust for contact-mechanical stimulation on the tympanic membrane (vibrating frequency of 69 kHz) and stimulation by airborne sound at best frequencies (for *a*, 16 kHz; for *b*, 10 kHz); for both types of stimuli rise- and decay-time 1 ms, plateau 20 ms, repetition rate 1 per s. Each data point represents 10 stimuli. The abscissa gives the stimulus intensity referred to relative voltage at the vibrating probe (for *a*, 0 dB ≙ 42 $\mathrm{mV_{pp}}$; for *b*, 0 dB ≙ 21 $\mathrm{mV_{pp}}$) and sound pressure levels in the free-field (for *a*, 0 dB ≙ 47 dB SPL; for *b*, 0 dB ≙ 27 dB SPL). The displacement of the tip of the probe (movement forwards and backwards is about 2,200 Å for 3 $\mathrm{V_{pp}}$ input voltage and linear over the whole operating range (calibrated by laser vibratometry). This magnitude of displacement corresponds to physiological values as measured on the cricket ear[12]. The tip of the probe was perpendicularly placed at a point on the membrane, about 200 μm ventromedial to a dark cuticular landmark (this position was maintained in all experiments). When the probe was not in contact with the membrane, even the highest voltage used elicited no response; this indicates that only contact-mechanical and no inadvertent acoustic stimuli were effective.

as the experiments presented in Fig. 1) observations were repeated for the corresponding unit on the other side of the CNS, maintaining both stimuli at 15 dB above threshold. In Fig. 2 the corresponding curves are shown for recordings from the right (solid line) and left (dashed line) interneurones. Since the two curves are nearly identical over the whole time axis, this example implies a symmetrical stimulus situation at both ears.

To deal with the second problem, we measured the response characteristics of several receptor cells and some interneurones, each stimulated with the piezotransducer and, after its removal, by airborne sound. As shown by the example in Fig. 3*a*, the receptor cell exhibited identical intensity and latency characteristics to the two stimuli (for similar results in moths see ref. 11). These data strongly suggest that the magnitude of neural excitation at the receptor can be finely controlled by both types of stimulus, which is important for the interpretation of Fig. 1 (see above). The coincidence between the two types of curve was found in both low- and high-frequency receptors. In Fig. 3*b* the corresponding curves for an auditory interneurone are shown. Although this interneurone exhibits a rather complicated intensity characteristic, both pairs of curves are similar. It is evident that at both neuronal levels, in the given circumstances, the contact-mechanical stimulation can be made equivalent to the acoustic free-field situation.

Referring to Fig. 1 we emphasise that by the contact-mechanical stimulation technique the binaural spike code can be separated from the binaural latency difference. The former code could be controlled by stimulus intensity (see Fig. 3), and the latter one was manipulated by experimental time delays between the two stimuli. Clearly both kinds of neuronal code control the activity of the interneurone. This is also true in free-field conditions, because with ipsilateral sound, the excitation of the ipsilateral receptors precedes inhibition from the contralateral side, which is also smaller in magnitude. In contralateral stimulus conditions this situation changes drastically, as now the greater inhibition precedes reduced excitation. Thus both cues—intensity and the time of arrival of the neural response in the CNS—have important, mutually reinforcing effects on synaptic transmission and ensure the optimal use of the peripherally processed directional information.

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Ionic basis of the receptor potential in a vertebrate hair cell

D. P. Corey & A. J. Hudspeth

Division of Biology, California Institute of Technology, Pasadena, California 91125

Vertebrate hair cells, the primary receptors of auditory, vestibular and lateral-line organs, occur in epithelia which separate fluids of differing ionic composition. The apical surfaces of hair cells, on which the mechanosensitive hair bundles are situated, face a high-K^+ fluid (termed endolymph in the inner ear); the basolateral surfaces instead contact fluid (perilymph or a related substance) of a composition similar to that of other extracellular fluids[1-3]. The universal occurrence of high-K^+ fluid on the apical surfaces of hair cells in vertebrates has been taken as evidence that it is important for the transduction process, in particular that it relates to the ionic specificity[4] of the conductance change[5] underlying the receptor potential. There is, however, conflicting experimental evidence regarding this specificity. K^+ has generally been thought to carry the receptor current, as replacement of endolymph with perilymph in the guinea pig cochlea abolishes the extracellularly recorded microphonic potential[6]. Yet microphonic potentials, as well as intracellular receptor potentials, have been recorded in other preparations when the apical surfaces of the hair cells faced instead a high-Na^+ saline, and thus when the electrochemical gradient for K^+ was near zero[5,7]. Ca^{2+} has also been proposed to carry the receptor current[8], but its concentration is quite low in endolymph[3], particularly that of the mammalian cochlea[9]. We present evidence here that the receptor current in a vertebrate hair cell is carried *in vivo* by K^+, but that the transduction channel is in fact nonspecific, being permeable to Li^+, Na^+, K^+, Rb^+, Cs^+, Ca^{2+}, and at least one small organic cation.

Hair cells were prepared for intracellular recording as described previously[5,10]. Sacculi were removed from adult bullfrogs and their otolithic membranes peeled away after loosening by mild proteolysis (incubation for 60 min with 0.03 mg ml^{-1} subtilopeptidase A, EC 3.4.4.16, at 22 °C). The tissue was positioned in an experimental chamber with standard saline on all cell surfaces and viewed with Nomarski differential interference contrast optics. Individual hair cells were penetrated by two glass microelectrodes, each 100–150 MΩ in resistance, connected to a simple voltage clamp circuit. To stimulate each impaled cell, its hair bundle was deflected with a fine glass probe (0.2 μm tip diameter) inserted horizontally between the kinocilium and the rest of the hair bundle. Probes were moved by an electronically controlled, two-dimensional stimulator, over a distance of 1–2 μm, with a 10-Hz, triangle waveform.

We first measured the current-voltage relationship for unstimulated hair cells (Fig. 1). The input resistance measured with two electrodes was 200–300 MΩ at a holding potential of −60 mV. Because the resistance was higher (200–900 MΩ) if measured with one electrode and an active bridge circuit, much of the apparent membrane conductance at this potential resulted from penetration damage. At membrane potentials more positive than about −50 mV, however, the resistance dropped to 6–7 MΩ. This conductance increase was largely blocked by 1 mM 3,4-diaminopyridine[11], and is evidently a voltage-dependent K^+ conductance. Such outward or delayed rectification may explain the rather small receptor potentials seen in some hair cell preparations[12,13]: because of the change in slope conductance, a receptor current which generates a receptor potential of 20 mV in bullfrog hair cells at a resting potential of −60 mV produces only 0.5 mV at −40 mV. Figure 1 shows an additional conductance increase, an inward or anomalous rectification, at potentials below −100 mV. Such a rectification has been hypothesised to explain certain aspects of electrical behaviour of the mammalian cochlea[14], but has not previously been demonstrated for vertebrate hair cells.

The stimulus-associated current, or receptor current, was measured by presenting periodic mechanical stimuli of saturating amplitude while clamping to various potentials. The receptor current at the holding potential of −60 mV ranged from 45 to 205 pA with a mean of 98 pA (±37 pA, s.d., $n = 28$ cells) and with the larger values more representative of healthy cells. Variation in the measured current was caused both by decline in the viability of a cell during prolonged recording and by mechanical drift of the stimulus probe with respect to the responsive range of the hair bundle. The voltage dependence of the receptor current was consequently determined by comparing the receptor current at a test potential to the current at the holding potential immediately before and after the potential step. The receptor current at −60 mV was normalised to 100 pA for each cell, and the receptor current at each test potential then scaled by the same amount. An implicit assumption in this analysis is that the mechanical sensitivity is not also voltage-sensitive, for instance that the responsive range of the hair bundle does not shift as the cell is depolarised.

A representative series of clamp records is shown in Fig. 2*a*. The receptor current reverses its direction from inward to outward at a membrane potential between −9 and +11 mV. Figure 2*b* combines normalised data from 24 cells; a linear regression fit to these data indicates a reversal potential of −2 mV. This is not the equilibrium potential for Na^+, K^+, or Ca^{2+}, since a standard, 124-mM Na^+ saline solution[5] faced all cell surfaces during these experiments. It could, however, result from a stimulus-dependent conductance that is nonspecific—that has a limited ability to discriminate among cations.

We consequently tested several cations using an *in vitro* microphonic technique[15]. The macular epithelium of the sacculus was positioned across a hole between two chambers, so as to separate them ionically and electrically. The potential across

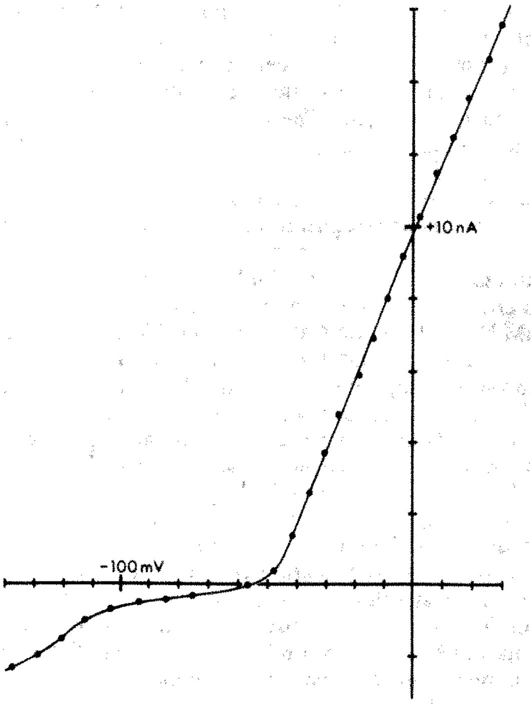

Fig. 1 Current-voltage relationship for a saccular hair cell, measured 50 ms after the start of a voltage step. Inward current is shown as negative. The holding potential was −60 mV. The striking outward rectification which occurs above −50 mV was blocked by 3,4-diaminopyridine and probably corresponds to the delayed rectification of most neurones. Inward or anomalous rectification is evident at potentials below −100 mV.

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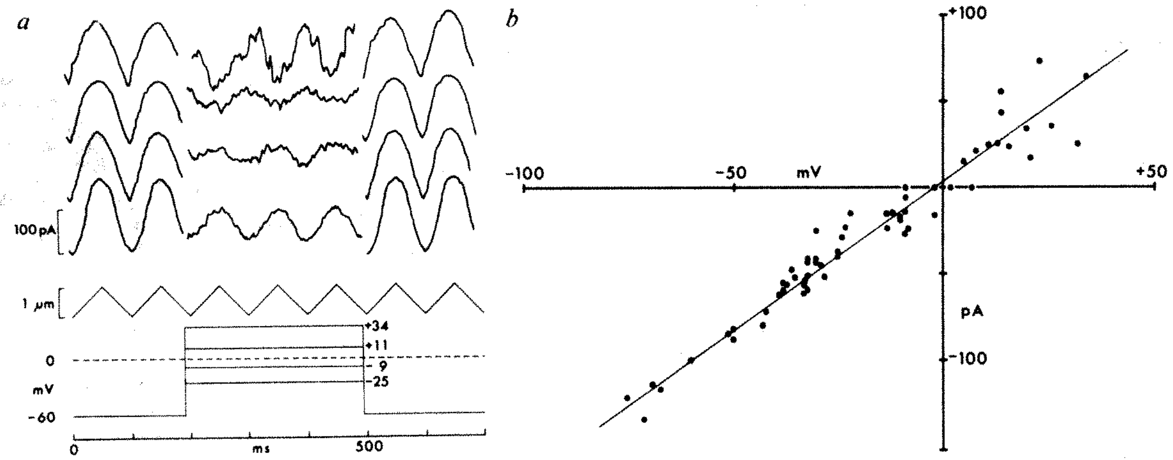

Fig. 2 *a*, Receptor currents at a holding potential of −60 mV and at test potentials of −25, −9, +11, and +34 mV; inward current is shown as negative. The membrane potential was held at −60 mV for 200 ms, clamped to the test potential for 300 ms, and returned to the holding potential. Each trace represents the average of 10 test steps. Ionic currents that were not stimulus-dependent were electronically subtracted before measuring the receptor current. The hair bundle was continuously moved with a 1.1-µm, 10-Hz, triangle-wave stimulus, shown below the current records; downward represents a deflection toward the kinocilium. *b*, Magnitude of the receptor current as a function of membrane potential; normalised data from 24 cells. The line is a linear regression fit to the data ($r^2 = 0.97$); its zero-current intercept, the reversal potential, is −2 mV. The external fluid was standard, high-Na^+ saline, so that the reversal potential is not the equilibrium potential of Na^+, K^+, or Ca^{2+}.

the epithelium was monitored with an electrode in each chamber; a second pair of electrodes was used to pass current to clamp the transepithelial potential at zero. Measuring the transepithelial current, rather than the transepithelial potential, avoided variations in response amplitude caused by slow changes in epithelial resistance. Hair cells were stimulated *en masse* by moving the otolithic membrane, which remained attached to the hair bundles, with a glass probe driven by a piezoelectric element. The stimulus was a saturating step displacement of amplitude 1.5 µm and duration 1 ms. The otolithic membrane can follow this fast stimulus, and the microphonic current turns on and off in much less than this time[15]. The charging time of the hair cell membrane is much longer, however, so that the intracellular potential should change only slightly during the pulse. The measured current is accordingly dependent on the resting potential and the permeability to the test ion, but not on voltage-sensitive conductances that are secondary to the receptor potential.

The chambers were separately perfused with a test solution on the apical side (130 mM of the relevant monovalent cation, 0.25 mM Ca^{2+}, 130 mM Cl^-, 3 mM D-glucose, 1 mM HEPES) and an artificial perilymph on the basolateral side (3.63 mM K^+, 122 mM Na^+, 1.36 mM Ca^{2+}, 0.68 mM Mg^{2+}, 130 mM Cl^-, 3 mM D-glucose, 1 mM HEPES). The apical surfaces of hair cells and supporting cells are bounded by tight junctions, which limit the exchange of ions between the two chambers.

All of the alkali cations supported the microphonic current. The mean relative signal amplitudes from eight experiments were: Li^+, 0.9; Na^+, 0.9; K^+, 1.0; Rb^+, 1.0; and Cs^+, 1.0. Ammonium ion (NH_4^+) was more permeant, with a relative amplitude of 1.3. To establish whether still larger ionic species could traverse the transduction channel, we bathed the apical surface with test solution containing 130 mM tetramethylammonium ion (TMA). This reduced the microphonic current to 0.2 of its value for 130 mM K^+, suggesting that TMA can pass through the transduction channel, although less readily than the alkali cations. To confirm this observation, TMA was tested on single cells using the intracellular voltage clamp method. The reversal potential, with 130 mM TMA on all cell surfaces, was −25 mV. Assuming that the extracellular TMA activity is roughly the same as the intracellular K^+ activity, this indicates a channel permeability to TMA about 0.4 that of K^+ (refs 16, 17). The fact that the permeabilities estimated by ionic current and by reversal potential are not equal suggests that TMA partially blocks the transduction channel[18].

The ability of one divalent ion, Ca^{2+}, to pass through the transduction channel was also demonstrated by extracellular recording. With 87 mM $CaCl_2$ in the test solution at the apical surface of the hair cell, the microphonic current was 0.3 that produced by 130 mM K^+. Because its concentration in endolymph is very low, Ca^{2+} can carry very little of the receptor current *in vivo*. Ca^{2+} does seem, however, to be a necessary cofactor for the response[5,8]: with a 130-mM K^+ saline, the microphonic current was abolished if the Ca^{2+} concentration was reduced below about 10 µM. Sr^{2+} replaces Ca^{2+} in this role, but Mg^{2+} and Ba^{2+} do not.

Because the transduction channel in frog saccular hair cells is nonspecific, the transduction current *in vivo* will be carried by cations approximately in proportion to their concentrations in endolymph. K^+, the predominant ion, will normally carry most of the current. The channel is equally permeable to the other alkali cations, however, and moderately permeable to Ca^{2+} and to an organic cation, TMA. As the unhydrated diameter of TMA is 0.54 nm, the channel interior must be at least this large. It therefore resembles the nonspecific, acetylcholine-activated channel of the motor endplate, which has an internal diameter of at least 0.65 nm (ref. 19).

It remains to be seen whether the transduction channels of all vertebrate hair cells are comparably nonspecific. The fact that Na^+ can support transduction in the goldfish sacculus[7] suggests that the channels in this organ are also nonspecific. The argument that ions other than K^+ cannot carry transduction current in the mammalian cochlea rests on the observation that substitution of Na^+ for K^+ in cochlear endolymph gradually but irreversibly blocks the response[6]. This might, however, reflect secondary effects of Na^+ accumulation on hair cells, such as the metabolic work required to pump this ion out of cells, rather than an inability of Na^+ to carry receptor current into cochlear hair cells. Inasmuch as the hair cells of lower vertebrates share with those of the cochlea a transduction process operating in high-K^+ fluid, mediated by stereocilia[10], and capable of great speed[15], it seems likely that the transduction channel of cochlear hair cells is also nonspecific in its ionic selectivity.

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Inhibition of platelet aggregation by native and desialised alpha-1 acid glycoprotein

Michael Costello, Barry A. Fiedel & Henry Gewurz

Department of Immunology, Rush University and Department of Microbiology and Immunology, University of Illinois at the Medical Center, Chicago Illinois 60612

The alpha-1 acid glycoprotein (orosomucoid; AAG) is a normal constituent of human plasma ($650 \pm 215\ \mu g\ ml^{-1}$) which increases in concentration as much as fivefold in association with acute inflammation and cancer, and thus is recognised as an acute phase protein[1,2]. AAG consists of a single polypeptide chain, has a molecular weight of 44,100, and contains ~45% carbohydrate including 12% sialic acid; it is the most negatively charged of the plasma proteins[3]. Certain of the biological properties of AAG are related to its sialic acid content[1,3]; thus, clearance and immunogenicity of AAG are markedly increased on desialisation[4,5]. The biological functions of AAG are largely unknown. AAG has the ability to inhibit certain lymphocyte reactivities including blastogenesis in response to concanavalin A, phytohaemagglutinin and allogeneic cells[6], and these inhibitory effects are enhanced in association with desialisation[7]. In view of these observations, a report that unphysiologically large (5–15 mg ml^{-1}) amounts of AAG inhibit the platelet aggregation induced by ADP and adrenaline[8], and evidence that a sialic acid-deficient species of AAG appears elevated in several chronic disease states[9,10], we compared the effects of AAG and its desialised counterpart (AAG-D) on platelet aggregation. We report that desialisation of AAG is associated with increased expression of activity inhibitory to the platelet aggregation otherwise observed on stimulation with ADP, collagen or thrombin.

AAG was isolated from human pleural fluids as described previously[6]. The purity of the four AAG preparations tested was established by SDS-polyacrylamide gel electrophoresis, immunoelectrophoresis and column chromatography on Sephadex G-75 and DEAE-cellulose. Desialisation was performed using *Vibrio cholerae* neuraminidase according to Schmid[11], with subsequent removal of the neuraminidase by reisolation of the AAG using DEAE-cellulose column chromatography; >75% loss of sialic acid[12] and 52% decrease of relative mobility on electrophoresis in polyacrylamide gels[13] were observed. Absence of residual neuraminidase activity in the AAG-D preparations was established by incubation with native AAG as the substrate using conditions optimal for enzyme activity; no neuraminidase activity was detected (<0.017 units per mg AAG-D). The standard platelet reaction mixtures consisted of 500 μl platelet-rich plasma (PRP), 200 μl of a dilution of AAG or AAG-D (in phosphate-buffered saline) and various amounts (in 10–15 μl) of the aggregating agent; the platelets were preincubated with AAG or AAG-D in the aggregometer for 30 s before stimulation with ADP, acid-soluble collagen[14], or thrombin. In experiments involving platelet activation by thrombin, 500 μl of a washed platelet suspension ($3.0 \times 10^8\ ml^{-1}$) was used in place of PRP[15].

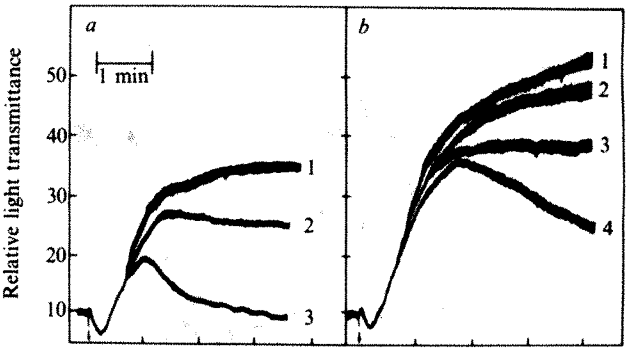

Fig. 1 Platelet aggregation induced by ADP at 12 μM (*a*) and 25 μM (*b*), and its inhibition by AAG and AAG-D. In *a*, curve 1 represents the buffer control; curve 2 inhibition by 2 mg ml^{-1} AAG; and curve 3 inhibition by 2 mg ml^{-1} AAG-D. In *b*, curve 1 represents the buffer control; curve 2 inhibition by 2 mg ml^{-1} AAG; curve 3 inhibition by 0.1 mg ml^{-1} AAG-D; and curve 4 the inhibition observed in the presence of 0.5–2.0 mg ml^{-1} AAG-D. AAG-D was substantially more inhibitory than the native molecule in all conditions tested.

The addition of either AAG or AAG-D (2 mg ml^{-1}) to PRP resulted in inhibition of the platelet aggregation otherwise observed on challenge with 12 μm ADP (Fig. 1*a*). AAG-D was substantially more inhibitory than native AAG at all doses of the glycoprotein tested, with inhibition directly proportional to the concentration of AAG and AAG-D and inversely proportional to the intensity of the ADP challenge stimulus. Thus, on challenge with a larger amount (25 μM) of ADP, 2 mg ml^{-1} AAG was no longer inhibitory, while AAG-D remained inhibitory even at concentrations as low as 50 μg ml^{-1}; concentrations of AAG-D to 2 mg ml^{-1} did not induce substantially greater degrees of inhibition but did bring about deaggregation of the stimulated platelets (Fig, 1*b*). The inhibitory effects of AAG and AAG-D were directed preferentially against the secondary wave of platelet aggregation, although with low (4 μM) ADP stimulation a minimal inhibitory effect on the primary aggregation wave also was observed.

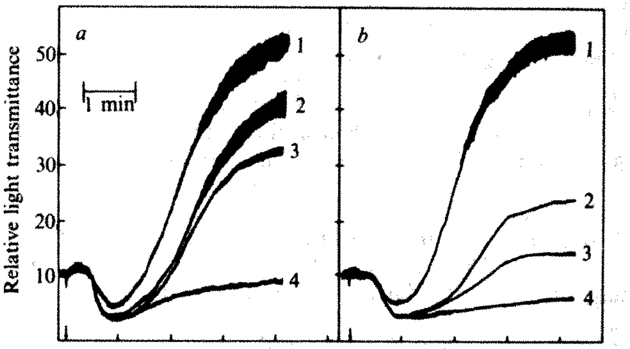

Fig. 2 Platelet aggregation induced by 9 μg ml^{-1} acid-soluble collagen and its inhibition by AAG (*a*) and AAG-D (*b*). In *a*, curve 1 represents the buffer control, while curves 2, 3 and 4 represent stimulation in the presence of 0.05, 0.5 and 2.0 mg ml^{-1} AAG, respectively. In *b*, curve 1 represents the buffer control, and curves 2, 3 and 4 show stimulation in the presence of 0.05, 0.1 and 0.5 mg ml^{-1} AAG-D, respectively. AAG-D again was substantially more inhibitory than the native molecule.

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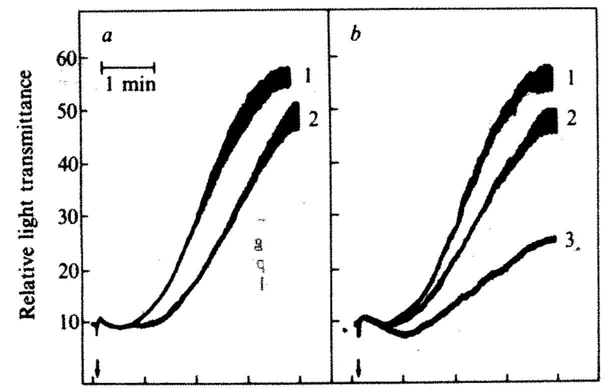

Fig. 3 Platelet aggregation induced by 0.014 U ml^{-1} bovine topical thrombin and its inhibition by AAG (*a*) and AAG-D (*b*). In *a*, curve 1 represents the buffer control, and curve 2 represents stimulation in the presence of 1 mg ml^{-1} AAG. In *b*, curve 1 represents the buffer control, and curves 2 and 3 show stimulation in the presence of 0.25 and 1 mg ml^{-1}, respectively. AAG-D again was substantially more inhibitory than the native molecule.

Both AAG and AAG-D were also capable of inhibiting the platelet response to collagen, with AAG-D again much more inhibitory than the native molecule (Fig. 2). Thus, AAG-D at 50–100 μg ml^{-1} markedly inhibited platelet aggregation, while native AAG was only minimally inhibitory at comparable concentrations; however, larger amounts of AAG (2 mg ml^{-1}) were fully inhibitory. Inhibition was characterised by decreased rates and extents of aggregation with a minimal effect on the onset of platelet shape change. The addition of AAG and particularly of AAG-D also resulted in decreased secretion of platelet serotonin on stimulation with ADP or collagen.

AAG and AAG-D also inhibited platelet aggregation induced by thrombin and AAG-D was again the more inhibitory with 250 μg ml^{-1}, compared to 1 mg ml^{-1} of native AAG, needed for significant inhibition (Fig. 3). Decreased inhibition was observed when the concentration of the thrombin challenge dose was increased. In controls for the above experiments platelet viability was unaltered as monitored by ^{51}Cr release, and thus could not account for the inhibitory effects observed. Further, the addition of neuraminidase in amounts (0.03–0.09 units ml^{-1}) up to fivefold greater than the minimum detectable in the assays used in this work had either no effect on, or enhanced, both ADP- and thrombin-induced platelet aggregation.

The mechanism(s) by which AAG inhibits the platelet response to ADP, collagen and thrombin is not yet known, nor is the manner in which desialisation seems to evoke further or enhance this inhibitory activity. Thus, removal of sialic acid residues may directly alter the exposure or effectiveness of appropriate reactive sites or indirectly favour a further critical modification of AAG such as its aggregation[16]. Moreover, we do not yet know the relative activities of the polymorphic or altered forms of AAG that have been observed in various sera and fluids[3,9,10]. Nonetheless, it is clear that both native and desialised AAG share with another acute phase protein, the C-reactive protein or CRP, the capacity to inhibit platelet responsiveness[14,15], and like CRP the effects of AAG are directed predominantly to events critical for the secondary wave of platelet aggregation. The present results thus point to AAG as a potentially significant modulator of platelet responsiveness, suggest desialisation or an associated alteration of the molecule as a mechanism by which this biological potential is evoked and/or enhanced, and support the hypothesis that the acute phase response represents, at least in part, a coordinated system to limit, modulate and/or otherwise direct host immune responses during periods of intense inflammation and tissue destruction.

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Cell-mediated immunity to *Theileria*-transformed cell lines

Terry W. Pearson* & Lena B. Lundin
International Laboratory for Research on Animal Diseases,
Nairobi, Kenya

Thomas T. Dolan & David A. Stagg
Veterinary Research Department, Kenya Agricultural Research
Institute, Muguga

In East and Central Africa the protozoan parasite *Theileria parva* causes a disease of cattle called East Coast fever (ECF). In Kenya alone between 60,000 and 85,000 cattle die from ECF every year[1]. Infected animals can recover from ECF either naturally[2] or after treatment with tetracyclines[3] or menoctone[4] and are subsequently able to resist challenge with the homologous strain of parasite. That this acquired resistance is due to cell-mediated rather than humoral immunity has been suspected[5,6] but never decisively shown. A major difficulty in studying immunity to ECF has been the lack of inbred animals for studying *Theileria*-specific immunity in the absence of allogeneic histocompatibility barriers. We have avoided this problem by measuring cell-mediated immune responses in a syngeneic system *in vitro*. Unidirectional mixed lymphocyte cultures (MLC) were set up using bovine peripheral blood lymphocytes (PBL) as responder cells and autologous cell lines transformed *in vitro* by *T. parva* as stimulator cells. In these cultures, DNA synthesis was induced in PBL from both normal and *Theileria*-immune animals. However, cytotoxic lymphocytes were induced only in cultures containing responder lymphocytes from *Theileria*-immune cattle. The results show that *Theileria*-transformed cells express antigens which are recognised by effector cells and provide evidence that cell-mediated cytotoxic mechanisms function in immunity to ECF.

By culturing cell suspensions from lymph nodes, spleen or peripheral blood of *Theileria*-infected cattle, transformed lymphoblastoid cell lines can be obtained[7]. Such cell lines can also be obtained by infecting normal bovine lymphocytes *in vitro* with *Theileria* sporozoites[8]. It is likely, therefore, that these cell lines represent the schizogonous stage of the parasite in the host animal which is characterised by the presence of macroschizonts in the transformed lymphocyte. Of more than 10 different *Theileria*-transformed cell lines examined by immunofluorescence, all were surface immunoglobulin negative and stained positively with fluoresceinated peanut agglutinin (G. E. Roelants *et al.* unpublished). Thus we believe that the *Theileria*-infected cells are bovine T lymphocytes[9].

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*Present address: Department of Biochemistry and Microbiology, University of Victoria, Victoria, British Columbia, Canada V8W 2Y2.

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Table 1 Stimulation of bovine lymphocytes by syngeneic *Theileria*-transformed cell lines

	Cell combination	Mean c.p.m.	Maximum stimulation ratio		Cell combination	Mean c.p.m.	Maximum stimulation ratio
A	PBL$_{784}$ × medium	117	—	*B*	PBL$_{951}$ × medium	241	—
	PBL$_{784}$ × PBL$_{784(R)}$	105	—		PBL$_{951}$ × PBL$_{951(R)}$	252	—
	LINE$_{784(R)}$ × medium	74	—		LINE$_{951(R)}$ × medium	286	—
	PBL$_{784}$ × LINE$_{784(R)}$	25,039	238.5		PBL$_{951}$ × LINE$_{951(R)}$	16,703	66.3
					PBL$_{989}$ × PBL$_{951(R)}$	5,061	21.0

Lymphocytes were separated from heparinised peripheral blood of female Friesian animals 951 and 784 by centrifugation through Ficoll–Paque (Pharmacia) and adjusted to 2.5×10^6 cells per ml in RPMI-1640 medium containing heat-inactivated fetal calf serum (5%), glutamine (2mM), 2-mercaptoethanol (5×10^{-5} M), penicillin (100 μg ml^{-1}), streptomycin (100 IU ml^{-1}) and HEPES buffer (10mM)[9]. Volumes of 0.1 ml were dispensed in triplicate into wells of Falcon 3040 flat-bottomed microplates. One hundred μl of medium alone or of a suspension of irradiated syngeneic *Theileria*-transformed cells (2.5×10^5 cells ml^{-1}) were added to the appropriate microwells. The subscript (R) indicates that the cells were irradiated with 5,000 R from a caesium source. The microplates were incubated at 37 °C in 5% CO_2 in air and at 48, 96, 144 and 192 h, 0.5 μCi ^{125}IUdR was added to each well. Sixteen hours later, cultures were collected onto glass fibre filters using a Skatron multiple sample harvester. Filters were dried under an IR lamp and counted for 1 min in a γ counter. Mean counts per minute were determined and stimulation ratios calculated as mean c.p.m. in PBL–syngeneic line combinations/mean c.p.m. in PBL–syngeneic PBL combinations. Stimulation ratios were determined for each time point but only the maximum stimulation ratios were shown here (peak was at day 6 for both experiments shown). In preliminary experiments, eight batches of fetal calf sera were tested at 5, 10 and 20% final concentration. One batch (used at 5% final concentration) gave the highest stimulation ratios and was used in all further experiments. Various cell concentrations, stimulator: responder cell ratios and irradiation doses were also tested and optimal conditions determined before starting the experiments presented in this paper. The data shown are representative of data obtained in four experiments performed with PBL and cell line from animal 784 and in six experiments with PBL and cell line from animal 951. The cell lines used as stimulators were derived by infecting lymphoid cells *in vitro* with sporozoites of *T. parva* from ground up ticks using the method of Brown *et al.*[8]. Lymph nodes were removed from Friesian heifers 951 and 784 and their cells infected from a common pool of *T. parva* (Muguga). The lymphoblastoid lines were maintained by twice weekly passage in Eagle's minimal essential medium with Earle's salts and 20% fetal calf serum. For use in MLC and for generation of cytotoxic lymphocytes, the cell lines were transferred and grown in RPMI-1640 medium containing 10% heat-inactivated fetal calf serum and were subcultured every two days to ensure healthy cells. All cattle used were judged to be healthy by the veterinary staff at the Kenya Agricultural Research Institute. Animals which were known to be *Theileria*-immune had high levels of anti-macroschizont antibodies (detected by indirect immunofluorescence) in their sera. Those which were considered non-immune were clinically normal and their sera contained no detectable anti-macroschizont antibodies.

It is thought that host cells must be infected with *Theileria* parasites before immunity can be achieved[10]. This led us to believe that the infected lymphocytes carry infection- or transformation-associated antigens on their surface and that these antigens are recognised by the host immune system. To test for such antigens, DNA synthesis was measured in MLCs containing syngeneic responder–stimulator combinations as shown in Table 1. *Theileria*-transformed cells stimulated ^{125}I-iododeoxyuridine (^{125}IUdR) uptake in autologous PBL from normal or *Theileria*-immune animals and with the same kinetics in each case. Normal PBL were stimulated to similar degrees with autologous and allogeneic *Theileria*-transformed stimulator cells (data not shown). The stimulation ratio in cultures containing the allogeneic PBL combination (PBL$_{784}$ × PBL$_{951(R)}$) was much lower than that obtained when the allogeneic *Theileria*-transformed cells were used as stimulators suggesting that the high stimulation was caused by special properties of the transformed cells and not simply by differences in normal histocompatibility antigens. Autoradiography showed that DNA synthesis was increased in the nuclei of responder lymphocytes (G. E. Roelants and T.P., unpublished). This stimulation was probably caused by antigens on the surface of viable stimulating cells since culture supernatants from MLCs had no effect on DNA synthesis of responder cells (Table 2). Addition of freshly prepared free macroshizonts to PBL cultures also failed to stimulate DNA synthesis. We did not detect schizonts in dividing cells in Giemsa stained preparations from 6–10 day MLCs showing that transformation of normal PBL had not occurred. Transformed cells, killed by heating or treated with the compound 993C (a 2-cycloalkyl-3-hydroxy-1, 4-naphthoquinone, Wellcome) to eliminate the schizonts, failed to stimulate DNA synthesis in MLC (Table 3). Compound 993C is selectively toxic for the macroschizont in the transformed cell and thereby stops parasite-induced proliferation but leaves the cells in a viable but non-transformed state[11]. These results suggest that antigens present on the surface of *Theileria*-transformed cells are involved in stimulation of normal syngeneic PBL and that the antigens are *Theileria*-associated and not due solely to culture *in vitro*. In addition, the heat-treatment experiment shows that viable stimulator cells are required, a feature of a true mixed lymphocyte reaction (MLR)[12].

Table 2 The effect of MLR culture supernatants on DNA synthesis in bovine lymphocytes

	Cell combination/supernatant	Mean c.p.m.	Stimulation ratio
A	PBL$_{951}$ + fresh medium	324	-
	+ supernatant 1	331	-
	+ supernatant 2	361	-
	+ supernatant 3	301	-
	+ supernatant 4	302	-
	+ supernatant 5	302	-
B	PBL$_{951}$ × LINE$_{951(R)}$ + fresh medium	2,568	12.7
	+ supernatant 1	2,201	10.9
	+ supernatant 2	2,201	11.4
	+ supernatant 3	1,999	9.9
	+ supernatant 4	2,109	10.5
	+ supernatant 5	1,990	9.9

Various cell combinations were set up in microplates following the procedure outlined in Table 1 legend. After incubation for 5 days, supernatants were removed from the culture wells and 100-μl aliquots (either neat or after concentrating fivefold with an Amicon UM-2 filter) were added to microwells containing normal PBL (*A*) or mixed lymphocyte cultures (*B*). Six days later, ^{125}IUdR was added to the microwells and the well contents collected as described in Table 1 legend. Supernatants were prepared from the following cell combinations: (1) PBL$_{951}$ × PBL$_{951(R)}$ (2) PBL$_{951}$ × LINE$_{951(R)}$ (3) PBL$_{951(R)}$ × LINE$_{951(R)}$ (4) LINE$_{951(R)}$ × medium (5) LINE$_{951}$ × medium. In experiment *B*, the control culture (PBL$_{951}$ × PBL$_{951(R)}$ showed 201 mean c.p.m.

Table 3 The effect of heating and drug treatment of *Theileria*-transformed stimulating cells in MLR

Cell combination	Mean c.p.m.	Stimulation ratio
PBL$_{951}$ × PBL$_{951(R)}$	340	1.0
PBL$_{951}$ × LINE$_{951(R)}$	2,860	8.4
PBL$_{951}$ × LINE$_{951(R)993C\ \mathrm{treated}}$	407	1.2
PBL$_{951}$ × LINE$_{951(R)\ \mathrm{heat\ treated}}$	238	0.7

Cell combinations were set up in microplates as described in Table 1 legend. Some stimulator cells were heat-killed (as assessed by Trypan blue exclusion) by incubating at 56 °C in a water bath for 45 min. Drug treatment of stimulator cells was performed by incubating 5×10^7 *Theileria*-transformed cells at 37 °C for 48 h with 1μg ml^{-1} final concentration of the compound 933C after which they were washed three times. The cells were 94% viable after treatment. The stimulation of DNA synthesis was assessed at days 3, 4, 5 and 6. Only the results from day 6 are shown here although the same pattern of stimulation was seen at all time points. The data shown are representative of data obtained in three experiments.

A positive MLR represents the cognitive and proliferative phase of an immune response[13]. However, proliferation of cells is not in itself evidence for functional immunity. For this reason, we tested for cell-mediated lysis of *Theileria*-transformed cell lines by using cells from MLCs as effectors in ^{51}Cr-release assays (Fig. 1). Cytotoxic activity was only detected after secondary stimulation *in vitro* of PBL from immune animal 713. Cytotoxic

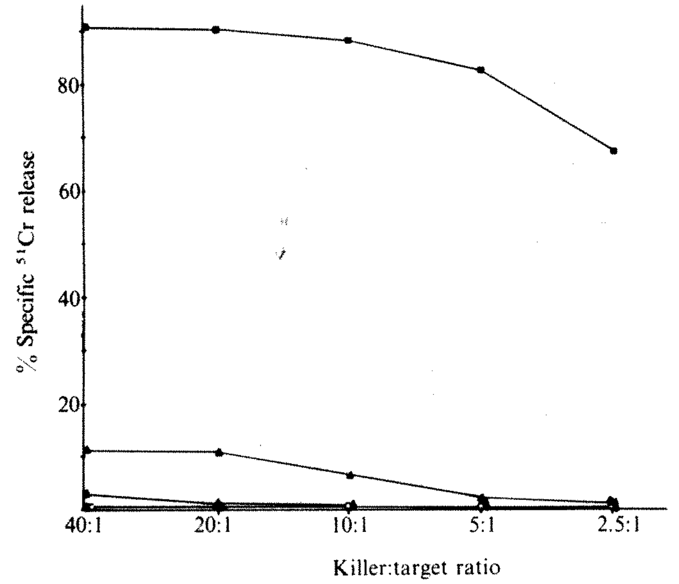

Fig. 1 Cell-mediated lysis of *Theileria*-transformed cell lines. Bovine peripheral blood lymphocytes were purified[9] from non-immunised animal 951 or from *Theileria*-immune animal 713 and cytotoxic lymphocytes were generated in MLC and assayed as previously described for the mouse[16], except that the stimulator cells were irradiated (5,000R from a caesium source), the MLCs were incubated for 6 days, and the cytotoxic cells were adjusted to 1×10^7 per ml. Animal 713 was immunised by infecting with *T. parva* sporozoites and treating with the compound 993C 11 days later. It was then injected at 2-month intervals with 1×10^9 cells of the autologous *Theileria*-transformed line 713 on three separate occasions. The *Theileria*-transformed cells were labelled with ^{51}Cr (ref. 17) and used as targets within 30 min of labelling. Background (medium) release was 9% for the LINE$_{713}$ and 10% for the LINE$_{951}$. The data shown are representative of data from nine independent experiments with effectors from PBL$_{713}$ and LINE$_{713}$ MLC combinations and four experiments using cells from PBL$_{951}$ and LINE$_{951}$ combinations.

Effector:

○ PBL$_{713}$ (directly out of animal) ⎫
△ PBL$_{713}$ × MEDIUM ⎬ On LINE$_{713}$ targets.
■ PBL$_{713}$ × LINE$_{713(R)}$ ⎭
● PBL$_{951}$ × LINE$_{951(R)}$ ⎫ On LINE$_{951}$ targets.
▲ PBL$_{713}$ × LINE$_{713(R)}$ ⎭

activity was also seen when effectors from PBL$_{A143}$ × LINE$_{A143.D7(R)}$ and from PBL$_{953}$ × LINE$_{953(R)}$ MLC combinations were used on homologous targets. The PBL$_{A143}$ were from a bovid which was infected with sporozoites and cured by treatment with tetracyclines and PBL$_{953}$ were from an animal which became naturally infected and was cured with menoctone. A low level of killing of allogeneic *Theileria*-transformed cell lines was observed with each of these effector populations. No killing was observed when effectors were from MLCs containing PBL from normal animals 951 (Fig. 1) or A149 (data not shown). It is likely that these PBL require an initial 'priming' in order to generate helper cells for cytotoxic cells or to generate cytotoxic cell precursors. In the generation of cytotoxic T lymphocytes in the murine system[14], proliferation of 'helper' or precursor lymphocytes occurs on initial exposure to antigen. It is possibly this type of proliferation that we measured in the primary MLRs. It follows that after secondary stimulation *in vitro*, as with PBL$_{713}$, PBL$_{A143}$ and PBL$_{953}$ which were taken from immune animals, enough 'helper' cells or cytotoxic cell precursors were present to allow differentiation of cytotoxic effectors.

Our results show that cytotoxic cells can be induced in PBL from *Theileria*-immune animals by co-culture with syngeneic *Theileria*-transformed cell lines. These cytotoxic cells kill *Theileria*-transformed cells and their generation may thus be used as a measure of immunity to the parasites. It is most likely that after challenge with *Theileria*, cytotoxic cells develop in both immune and non-immunised cattle. Why then do most non-immunised cattle die after between 18 and 24 days of infection? Our results support the widely held hypothesis[15] that it is simply due to outpacing of the immune response by growth of the parasite since once an animal has recovered from infection it is solidly immune. By biasing the immune system towards *Theileria*-specific cell-mediated immunity it should be possible to immunise cattle. Our results suggest that priming of the bovine immune system with allogeneic *Theileria*-transformed cell lines may be possible, that is, the priming may not be H-restricted. If such cell lines are attenuated so that they do not cause lethal infection they may allow sensitisation of the immune system so that a second exposure to antigen causes an effective cytotoxic response to *Theileria*-transformed lymphocytes.

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Clindamycin and lincomycin alter miniature endplate current decay

J. F. Fiekers, I. G. Marshall* & R. L. Parsons

Department of Physiology and Biophysics, College of Medicine,
University of Vermont, Burlington, Vermont 05405

Antibiotic-induced muscle paralysis has frequently been found in both experimental animals and man with three distinct classes of antibiotic: (1) streptomycin and related aminoglycoside compounds, (2) polymyxins and (3) tetracyclines[1]. Recently lincomycin and its chemical congener, clindamycin, have been reported to produce muscle paralysis which has different characteristics from those seen with other classes of antibiotic[2–4]. Although closely related in chemical structure, lincomycin and clindamycin also seem to produce muscle paralysis by different mechanisms. Clindamycin is considered to exert a direct depressant action on muscle contractility whereas the action of lincomycin is considered to be primarily a depression of neuromuscular transmission. We report here that each of these antibiotics had a significant but different influence on endplate channel behaviour. Clindamycin increased the rate of miniature endplate current (m.e.p.c.) decay and reduced its voltage sensitivity without altering its exponential nature. Lincomycin split m.e.p.c. decay into an initial rapid phase followed by a prolonged phase.

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*Present address: Department of Physiology and Pharmacology, University of Strathclyde, Glasgow, UK.

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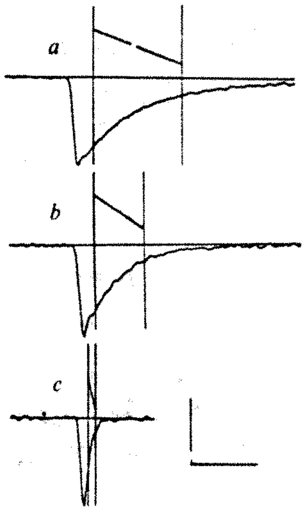

Fig. 1 The effect of clindamycin on m.e.p.c. decay is concentration dependent. Averaged m.e.p.cs (13–17 individual currents) obtained in a fibre voltage clamped at −120 mV in control Ringer (*a*) 2.12×10^{-5} M clindamycin (*b*) and 4.26×10^{-4} M clindamycin (*c*). Above each current record a portion of the decay is plotted semilogarithmically and fitted with a least-squares regression line to evaluate the decay time constant. The linearity of the plot indicates that m.e.p.cs decay as a single exponential with time. The decay time constants (ms) were: *a*, 3.41; *b*, 1.89; *c*, 0.298. Calibration bars: *a*, 5.4 nA, 3.5 ms; *b*, *c*, 2.7 nA, 3.5 ms.

Spontaneous m.e.p.cs were recorded in voltage-clamped twitch fibres of the costocutaneous muscle of garter snakes (*Thamnophis* sp.). Muscles were removed with a portion of the rib and scale on which they inserted and mounted in a small (4 ml) Plexiglass chamber filled with physiological solution of composition (mM): NaCl 159; KCl 2.15; $CaCl_2$ 2.0; $MgCl_2$ 4.2; HEPES 1.0, at *p*H 7.3–7.4. Experiments were carried out at room temperature (20–24 °C). All solutions contained 100 nM tetrodotoxin. The snake neuromuscular junction is well suited for these studies, for the endplate region is compact relative to the fibre space constant (1–4 mm)[5], enabling the voltage clamp to provide effective voltage control over the entire active synaptic area. With the muscle spread laterally, individual endplates can be easily visualised and voltage clamped with two microelectrodes as described previously[6]. M.e.p.cs were digitally recorded and analysed using a DEC PDP8E computer; those with the most rapid rate of rise (<1 ms) were selected for analysis. An average of 10–20 m.e.p.cs were recorded and averaged at each holding potential. Stock solutions of the antibiotics (clindamycin HCl, lincomycin HCl) were freshly prepared in the physiological solution. Drugs were added by replacing the bath volume three times with drug-containing solution. An equilibration period of at least 10 min elapsed before recording at each concentration. Averaged control m.e.p.cs could be fitted to the expression, $I(t) = I(0) \exp(-t/\tau)$, where $I(0)$ is the peak current, t is time after peak and τ is the decay time constant given by the semilogarithmic slope. At all voltages control m.e.p.cs decayed with a single exponential time course. The decay time constant was determined by confining the analysed portion to between 20 and 80% of the peak amplitude.

Clindamycin reduced the amplitude and shortened the time course of m.e.p.c. decay. The effects of clindamycin were concentration dependent in the range studied (2.12×10^{-5} M–4.26×10^{-4} M). Figure 1 shows the concentration dependence in a single fibre voltage clamped at −120 mV. Note that both in control records and records of the two concentrations of clindamycin the m.e.p.cs decayed as a single exponential with time. This was consistently observed at all voltages and concentrations of clindamycin. The dependence of the m.e.p.c. decay on clindamycin concentration is indicated by the amount of change in the semilogarithmic plot above each current record. In Fig. 1 τ was decreased 45% and 91% in clindamycin concentrations of 2.12×10^{-5} M and 4.26×10^{-4} M, respectively.

In normal physiological solution $\tau_{\text{m.e.p.c.}}$ is sensitive to changes in membrane potential[6–8]. The voltage dependence of $\tau_{\text{m.e.p.c.}}$ can be accurately described by the exponential function

$$\tau(V) = \tau(0)e^{-AV} \tag{1}$$

where $\tau(0)$ is the decay time constant at zero membrane potential, V is the membrane potential and A is the voltage coefficient of τ. In five control cells the average coefficient of voltage sensitivity, A (mV^{-1}), was 0.015 ± 0.001 (mean ± s.e.). This value is similar to that previously reported for snake twitch fibres[6]. The dependence of τ on voltage obtained in a single fibre for both control and two concentrations of clindamycin is illustrated in Fig. 2. Values of $\tau(0)$ and A calculated according to equation (1) were: control physiological solution: $\tau(0) = 0.783$ ms, $A = -0.0118\ \text{mV}^{-1}$; 2.12×10^{-5} M clindamycin: $\tau(0) = 0.775$ ms, $A = -0.0069\ \text{mV}^{-1}$; 4.26×10^{-5} M clindamycin: $\tau(0) = 0.521$, $A = +0.0033\ \text{mV}^{-1}$. In the presence of clindamycin there was a concentration-dependent reduction in the voltage sensitivity. With concentrations of clindamycin of 2.12×10^{-4} M or higher the voltage sensitivity was either abolished or reversed (as in Fig. 2). The shortening of m.e.p.c. decay and reduction in the voltage sensitivity of $\tau_{\text{m.e.p.c.}}$ produced by clindamycin were reversible on return to control physiological solution.

In marked contrast to the effects of clindamycin on m.e.p.c. decay lincomycin converted the decay of m.e.p.cs to a multiexponential function consisting of at least two distinct components, one more rapid and another slower than the decay of control m.e.p.cs. The effect of lincomycin on m.e.p.c. decay rates and amplitude was both voltage and concentration

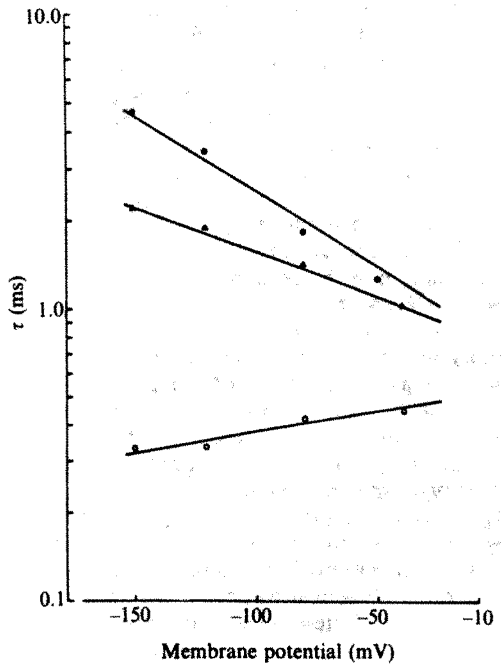

Fig. 2 The voltage dependence of τ is reduced by clindamycin. m.e.p.c. decay for both control and clindamycin depend exponentially on voltage over the range −40 mV to −150 mV. Control (●) and measurements in the presence of clindamycin, 2.12×10^{-5} M (▲) and 4.26×10^{-4} M (○), were fitted according to equation (1).

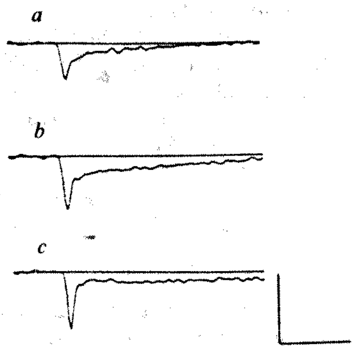

Fig. 3 The action of lincomycin on m.e.p.c. decay is voltage dependent. Averaged m.e.p.cs (13–20 individual currents) were recorded in a single fibre at −50 mV (*a*), −100 mV (*b*) and −150 mV (*c*). Lincomycin concentration was 4.43×10^{-4} M. Calibration bars: 2.7 nA, 3.5 ms.

dependent. Increasing the concentration of lincomycin above the threshold concentration (10^{-4} M) at a fixed voltage produced an increase in the relative size of the rapid initial decay and prolonged the time course of the terminal phase. Similarily, these phases varied with membrane potential at a constant lincomycin concentration. The voltage dependence of lincomycin action recorded in a single voltage-clamped fibre is shown in Fig. 3. The initial fast component of decay declined more quickly and comprised a larger portion of the total m.e.p.c. decay as the membrane was hyperpolarised. The slow component became greatly reduced in amplitude and decayed very slowly with hyperpolarisation. The time constants of decay for both phases were determined using a computerised least-squares exponential fitting program[9]. For the currents depicted in Fig. 3 the fast and slow decay time constants (ms) were, respectively, 0.30 and 3.56 at −50 mV, 0.17 and 6.78 at −100 mV, 0.17 and 20.8 at −150 mV.

The time constant of m.e.p.c. decay is well established as a measure of the mean open duration of endplate channels in frog twitch fibres[7]. A similar relationship has been reported for snake twitch fibres[6]. Alteration in the decay of both e.p.cs and m.e.p.cs have been reported for various drugs, including local anaesthetics[10–12] and belladonna alkaloids[13,14]. The results reported here for clindamycin are similar to those previously reported for atropine and the lignocaine derivative QX314 (refs 10, 11). In addition, the splitting of m.e.p.cs by lincomycin is qualitatively similar to results reported for scopolamine[13] and the lignocaine derivative QX222 (refs 10, 11). These similarities are consistent with an interaction of the antibiotics with the open state of the acetylcholine receptor–channel complex, creating a channel with little or no conductance[12,15].

Clindamycin differs from lincomycin in chemical structure by the presence of a chloride substituent in position 7. The present results indicate that this chemical modification, which greatly increases the lipid solubility, influences the characteristics of m.e.p.c. decay. Of particular importance is the finding that these agents modify channel behaviour in concentrations which might normally be present in plasma. We suggest that the interaction of antibiotics with the endplate receptor–channel complex might help to explain the differences in neuromuscular block obtained with these antibiotics and also improve our understanding of the mechanisms of antibiotic-induced paralysis.

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Role of intracellular Ca^{2+} sequestration in β-adrenergic relaxation of a smooth muscle

Erich Mueller & C. van Breemen

Department of Pharmacology, University of Miami School of Medicine, Miami, Florida 33101

Various mechanisms have been proposed for β-adrenergically mediated relaxation of smooth muscle. All theories suggest the involvement of cyclic AMP as a second messenger: β-agonists stimulate adenylate cyclase which converts ATP to cyclic AMP[1] and protein kinase, activated by cyclic AMP, is then thought to catalyse a protein phosphorylation that leads to a reduction in free Ca^{2+}, thus effecting relaxation[1]. How this last step is accomplished is much debated, but the following possibilities are currently considered as the mechanisms responsible for cyclic AMP-induced reduction of cytoplasmic Ca^{2+}: activation of a Ca^{2+}-ATPase in the plasma and/or sarcoplasmic reticulum membranes which lowers cytoplasmic $[Ca^{2+}]$ in a direct manner or stimulation of $(Na^+$–$K^+)$ATPase in the cell membrane which may indirectly effect Ca^{2+} extrusion[2–8]. Among the hypotheses suggested, those of Ca^{2+} sequestration by the sarcoplasmic reticulum and of Ca^{2+} extrusion across the cell membrane are consistent with each other if it is assumed that both processes are effected by a cyclic AMP-sensitive Ca^{2+}-ATPase. However, quite a different mechanism is implied by involving the Na^+–K^+ pump and Na^+–Ca^{2+} exchange carrier. In this report, we present evidence that suggests intracellular Ca^{2+} sequestration is the mechanism involved.

Stopping the Na^+–K^+ pump with ouabain initiates a transient contraction in the taenia coli. The inhibited pump is unlikely to be stimulated by β-adrenergic activation, so this system provides the opportunity to test whether $(Na^+$–$K^+)$ATPase is required in cyclic AMP-mediated relaxation. The possible involvement of a Na^+–Ca^{2+} exchange carrier can be tested by studying relaxation of a high K^+-induced contracture in the absence of Na^+. In Fig. 1*a*, β-stimulation, phosphodiesterase inhibition and the application of 8-bromo-cyclic AMP can be seen to relax the ouabain-induced contraction. Each of these has been postulated to relax smooth muscle by increasing protein kinase activity through a rise in cytoplasmic cyclic AMP. In each case, the muscle relaxed below the resting tension. The delayed response of 8-bromo-cyclic AMP may be due to its slower diffusion into the cell. It is unlikely that these results are due to incomplete blockade of the Na^+–K^+ pump because they were duplicated with a contraction initiated by 10^{-4} M ouabain in the absence of external K^+.

Each of these agents also relaxed 145 mM K^+/0 Na^+ contractures (Fig. 1*b*), although the isoprenaline relaxation was transient. The decline in tissue Na^+ was measured in the 145 mM K^+/0 Na^+ solution. After exposure for 0, 2, 5 and 10 min, respective tissue Na^+ concentrations were 60 ± 3, 14.4 ± 1.2, 4.9 ± 0.9 and 0 mM Na^+ per kg taenia coli. At the point

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when relaxing agents were added (3 min), not all the Na$^+$ had left the cells. However, depletion of extracellular Na$^+$ reversed its gradient and would thus be unable to effect Ca^{2+} extrusion as postulated by Scheid *et al.*[7]. Identical relaxations were also obtained after exposure to the 145 mM K$^+$/0 Na$^+$ solution for 30 min, when all Na$^+$ had left the tissue.

Net Ca^{2+} fluxes (Fig. 2*a*, *b*) were measured during phosphodiesterase inhibition, as R0-20-1724 (a phosphodiesterase inhibitor; Roche) induced the most rapid tension reductions (see Fig. 1*b*). Neither ouabain nor the subsequent addition of R0-20-1724 induced significant changes in total exchangeable tissue Ca^{2+} (Fig. 2*a*). These results indicate that Ca^{2+} is released intracellularly during ouabain contraction and sequestered by an intracellular store during phosphodiesterase-induced relaxation.

The high-K$^+$ contraction (Fig. 2*b*) is characterised by an initial phasic portion that has been postulated to be at least partly mediated by intracellular Ca^{2+} release[9,10]. This is followed by a tonic contracture maintained by a net influx of Ca^{2+}. Again, however, no change in Ca^{2+} is observed with relaxation.

Relaxation by all three procedures, which raise cellular cyclic AMP, has been shown to occur when the Na$^+$–K$^+$ pump is inoperative or when there is no Na$^+$ in the medium or the cells. These results indicate that an increased inward-directed Na$^+$ gradient, whether or not the result of Na$^+$–K$^+$ pump stimulation, is not essential for β-adrenergic relaxation.

A recent study[7] has reported that Na$^+$–K$^+$ pump inhibition decreased the relaxing effect of isoprenaline or carbachol contractions. This could also be explained by superimposition of the ouabain contraction on the contractile effect of the carbachol.

The data presented here support the hypothesis that cyclic AMP-mediated relaxation is caused by Ca^{2+} sequestration[4,11,12]. The evidence also suggests that contraction is at least partially regulated by intracellular Ca^{2+} stores. Although the ouabain-induced myofilament activation seems to be controlled primarily by these stores, the high K$^+$ depolarisation produces activation that depends largely on extracellular Ca^{2+}. Finally, note should be made of recent evidence[13] that cyclic AMP may effect relaxation by inactivating myosin light chain kinase, thus reducing tension at the myofilament level. The data presented here are equally consistent with this model and that of stimulated sarcoplasmic reticulum Ca^{2+}-ATPase.

Either or both of these mechanisms may be operative in taenia coli during β-mediated relaxation but our evidence rules out net efflux of Ca^{2+}, specifically that by a Na$^+$–Ca^{2+} exchange carrier.

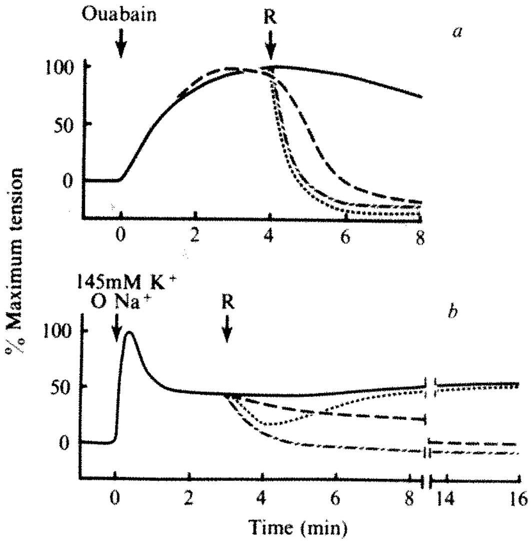

Fig. 1 *a*, Relaxation of ouabain-induced contractions. Tension of pieces ot taenia coli ~1 cm long was measured isometrically in physiological saline solution (PSS) (in mM; NaCl, 140; KCl, 5; CaCl$_2$, 1.5; MgCl$_2$, 1; dextrose, 10; HEPES, 5; *p*H 7.2, oxygenated) to which atropine (10^{-6} M) and phentolamine (10^{-5} M) had been added. These blocked any possible muscarinic or α-adrenergic effects, respectively. Muscles were contracted with 2×10^{-5} M ouabain. Four minutes later the relaxants were added: 5×10^{-4} M 8-bromo-cAMP (– – –), 10^{-3} M R0-20-1724 (– · –) or 10^{-5} M isoprenaline (· · · · ·). *b*, Relaxation of high K$^+$-induced contractions. Procedure as for *a* except that taenia were contracted in PSS in which all Na$^+$ was replaced by K$^+$. Relaxants were added 3 min after t_0.

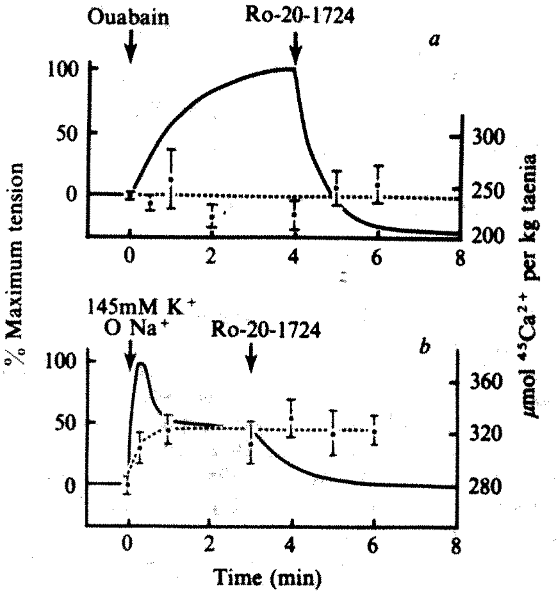

Fig. 2 *a*, Net Ca^{2+} content of taenia coli during ouabain-induced contracture and subsequent R0-20-1724-induced relaxation. Tissues were incubated in ^{45}Ca-labelled PSS for 90 min to label tissue Ca^{2+}; they were then sequentially exposed to the contracting and relaxing solutions as in Fig. 1 which were labelled at the same specific radioactivity. Labelled Ca^{2+} was measured in a scintillation counter after removal of extracellular Ca^{2+} in ice-cold PSS (0 Ca + 2 mM EGTA) for 45 min. Points represent mean ± s.e. for four determinations. *b*, Net Ca^{2+} during high K$^+$-induced contraction and subsequent relaxation by R0-20-1724. Tension tracing from *a*. Tissues were placed in 145 mM K$^+$–0 Na$^+$ HEPES buffer and R0-20-1724 (1 mM) was added 3 min later. ^{45}Ca measurements were made as in *a*.

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Extracellular but not intracellular application of peptide hormones activates pancreatic acinar cells

H. G. Philpott & O. H. Petersen

Department of Physiology, University of Dundee, Dundee, UK

Peptide hormones, like neurotransmitters, are traditionally thought to activate cells by interacting with receptor sites accessible only from the extracellular space[1-3]. However, there is no available evidence that establishes whether intracellular injections of peptide secretagogues can or cannot initiate cell activation. In view of recent demonstrations that peptide hormones can penetrate the intracellular space in some tissues[1,4] and the reports that intracellular injections of the neurotransmitter, dopamine, into acinar cells of cockroach salivary gland cause stimulation[5] it seems of fundamental importance to test directly whether introduction of peptide secretagogues inside acinar cells of mammalian exocrine tissue can induce cell activation without first interacting with the outer surface of the external cell membrane. The data presented here show that injections of the secretagogue peptides caerulein and bombesin-nonapeptide (bombesin-NP) into pancreatic acinar cells fail to evoke the characteristic potential and conductance changes that are observed following extracellular applications of these peptides.

The experiments were carried out on segments of mouse pancreas superfused with a physiological saline solution equilibrated with 95% O_2, 5% CO_2 at 37 °C. Membrane potentials and resistance from two electrically coupled acinar cells were measured with two microelectrodes and application of caerulein and bombesin-NP from microelectrodes using ionophoresis was carried out as previously described[6,7]. One intracellular microelectrode was filled with 3 M KCl and the other with 30 μM caeruletide (diethylamine salt) dissolved in 0.9% NaCl (*p*H 4) or 100 μM bombesin-nonapeptide hydrochloride in 0.9% NaCl (*p*H 4) (Farmitalia). The extracellular micropipette was filled with the same peptide solution as that used for the intracellular electrode.

The effects of caerulein ionophoresis are shown in Fig. 1. Extracellular application evoked the characteristic cell depolarisation and reduction in input resistance from the recording cell. In contrast, after the caerulein electrode had been inserted into a communicating acinar cell, intracellular ionophoresis had no effect on the electrical properties of either of the two impaled cells, while extracellular caerulein application from another electrode still evoked the characteristic response. After withdrawal of the tip of the intracellular caerulein electrode to the extracellular space, the electrode was still able to deliver caerulein and thereby effect the usual electrical response from the cell that remained impaled with the KCl electrode. From the 32 intracellular ejections of caerulein that were carried out on 14 acinar cells in pancreatic tissue from five mice, no response from the cells was obtained. Of special interest in Fig. 1 is the effect of spontaneous leakage of caerulein from the caerulein electrode causing depolarisation of the recording cell[7]. This was abolished once the caerulein electrode had been impaled into an acinar cell, allowing both cells to repolarise.

Figure 2 demonstrates that intracellular ionophoresis of bombesin-NP has no effect on the electrical properties of the acinar cell membrane in conditions where extracellular bombesin-NP applications evoked clear membrane resistance and potential changes. Application of a negative current through an extracellular bombesin electrode does not release bombesin or cause stimulation, but application of a positive ejection current does achieve this. The membrane depolarisation effect observed after applying a negative, hyperpolarising

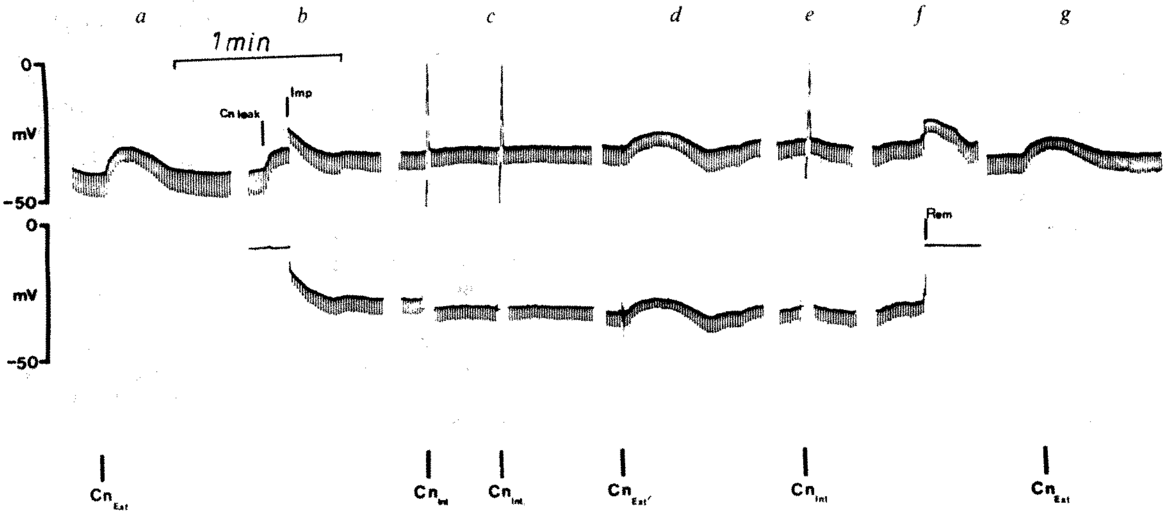

Fig. 1 Effect of extracellular and intracellular ejection of caerulein, applied to pancreatic acinar cells, on the membrane potential and input resistance. Panels *a*–*g* are consecutive traces. In *a* the cell membrane potential was measured from an intracellular KCl microelectrode through which rectangular hyperpolarising current pulses (2×10^{-9} A, 100 ms duration) were repeatedly injected to obtain cell input resistance. At $\mathrm{Cn_{Ext}}$ a positive ionophoretic current (100 nA, 500 ms duration) was applied to a caerulein-containing electrode (Cn-electrode) whose tip was positioned near to the surface of the impaled cell. In *b* the Cn-electrode, previously used for extracellular ionophoresis, was impaled at 'Imp' into an acinar cell that was in electrical communication with the cell impaled with the KCl electrode. 'Cn-leak' indicates the depolarisation of the recording cell arising from caerulein leakage from the approaching tip of the caerulein electrode, before impalement. In *c* and *e* at places marked '$\mathrm{Cn_{Int}}$' intracellular ionophoresis of caerulein was carried out. At *d* a second caerulein electrode was positioned near to the two indwelling electrodes and used for extracellular caerulein ionophoresis at '$\mathrm{Cn_{Ext'}}$'. In *f* the intracellular caerulein electrode was removed from the acinar cell at the place marked 'Rem'. In *g* after time was allowed for the first impaled cell to recover from disturbance caused by removal of the caerulein electrode, the same caerulein electrode was then used for extracellular ionophoresis at '$\mathrm{Cn_{Ext}}$'. A positive injection current of 100 nA lasting 500 ms was used throughout the experiment for caerulein ionophoresis. Time intervals between panels *a* and *b*, 20 s; *b* and *c*, 40 s; *c* and *d*, 6 min; *d* and *e*, 60 s; *f* and *g*, 20 min.

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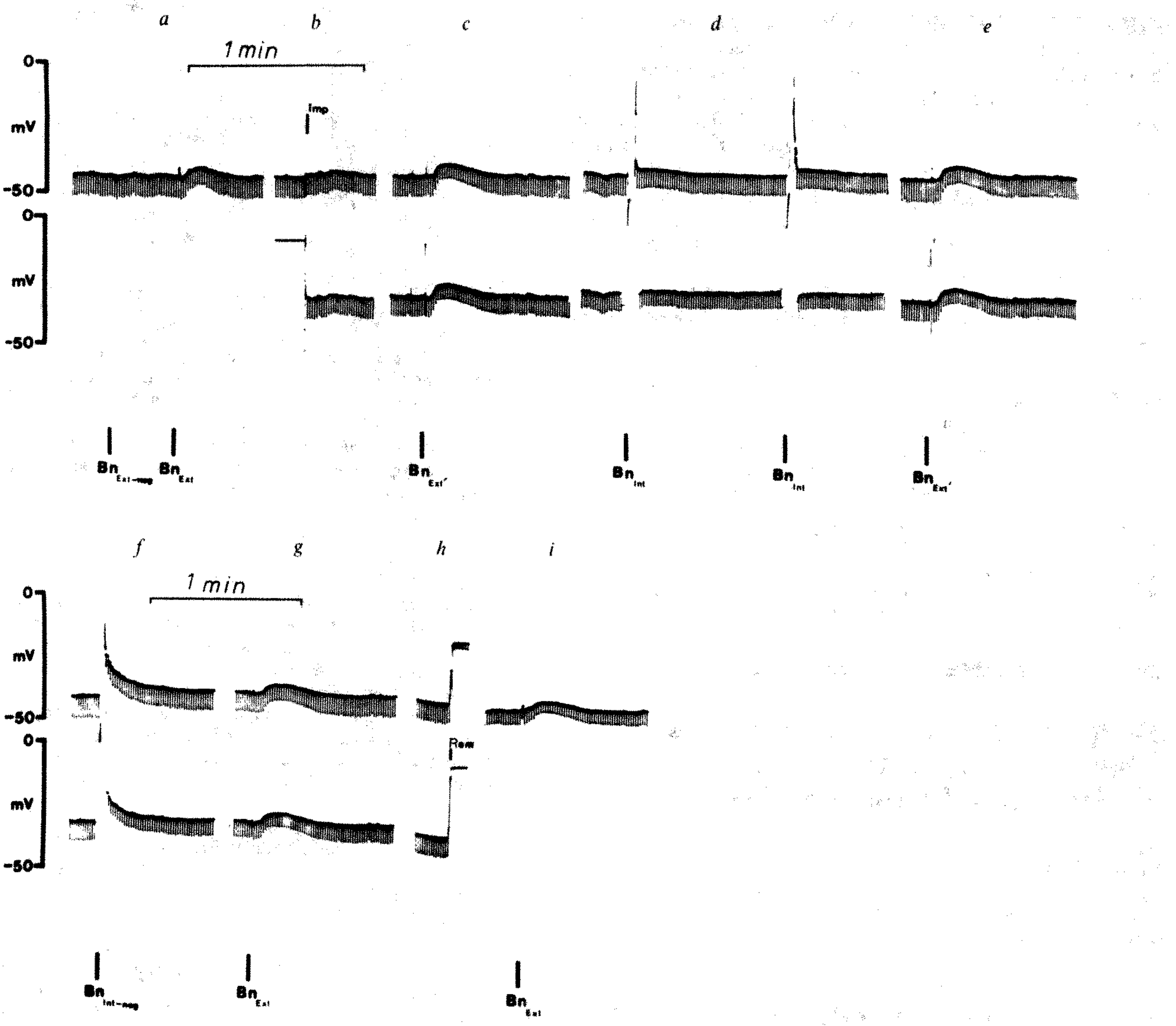

Fig. 2 Effect of extra- and intracellular ionophoresis of bombesin on pancreatic acinar cells. At 'Bn$_{\text{Ext-neg}}$', a negative ejection current was applied to a bombesin-containing electrode, near to the surface of the impaled cell. A second but positive current was then applied at 'Bn$_{\text{Ext}}$'. In *b* the Bn-electrode was inserted into an acinar cell coupled with the first recording cell. In *c* and *e* another extracellular bombesin electrode was placed near to the coupled cells and at 'Bn$_{\text{Ext'}}$' an ionophoretic current was then applied. In *d* at places marked 'Bn$_{\text{Int}}$' intracellular ionophoresis of bombesin was carried out. In *f* at 'Bn$_{\text{Int-neg}}$' the effects of passing a hyperpolarising current through the intracellular bombesin electrode are shown. *h*, Bn electrode removed at 'Rem'. *i*, At 'Bn$_{\text{Ext}}$' bombesin was ionophoresed extracellularly on to the remaining impaled cell using the previously indwelling Bn electrode. Throughout the experiment an ejection current of 400 nA lasting 500 ms was used for bombesin-NP ionophoresis. Time intervals between panels *a* and *b*, 7 min; *b* and *c*, 2 min; *c* and *d*, 7 min; *d* and *e*, 3 min; *e* and *f*, 7 min; *f* and *g*, 50 s; *g* and *h*, 13 min; *h* and *i*, 7 min.

current through the intracellular bombesin electrode (Fig. 2*f*) is not, therefore, due to bombesin-induced effects, but results from breakdown in the dielectric properties of the membrane, usually associated with intracellular injection of large hyperpolarising currents[8]. Of the 37 intracellular injections of bombesin into 11 acinar cells obtained from four mice, 30 showed no effect on membrane potential and resistance, 5 results showed a small effect on membrane resistance but no depolarisation, and 2 results gave a small but definite depolarisation and resistance reduction when compared to the bombesin-evoked responses obtained with extracellular application using the same electrode. The exceptional intracellular bombesin depolarising effects were only observed in cells where the resting membrane potentials were extremely low (< -23 mV). These effects are likely to have arisen from seepage of bombesin out of the acinar cell due to incomplete resealing of the cell membrane around the impaling electrode. Evidence for the leakage of intracellularly applied calcium out of injected cells with very low membrane potentials, has previously been given[9].

The experiments presented here are the first to monitor the electrical response of target cells during intracellular application of peptide hormones. The results provide evidence for the hypothesis that peptides like caerulein and bombesin, as also found for the neurotransmitter acetylcholine[2,3], attach to receptors on the outer surface of cell membranes as a prerequisite to stimulation. Specific binding of radiolabelled bombesin to the plasma membrane of pancreatic acinar cells has recently been demonstrated[10].

There seems to be a discrepancy between the results reported here and the study of House *et al.*[5] showing potential changes in cockroach salivary gland cells, with intracellular dopamine application. The apparent intracellular dopamine effects, however, as the authors suggested[5], may have been due to leakage of injected transmitter into the extracellular compartment.

Having established that, in order to achieve cell activation, the pancreatic acinar secretagogues must first attach to receptors on the extracellular side of the plasma membrane, there seems to be a need for an intracellular mediator. Intracellular Ca^{2+} application has been shown to mimic the electrical effects induced by outside application of pancreatic secretagogues[8,9] and therefore seems the most likely intracellular messenger. An alternative but more complicated theory is that a further mediation step may occur and take the form of a peptide hormone/receptor complex undergoing cell internalisation. To test this possibility studies should be carried out in the pancreas.

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Morphine-induced striatal dopamine efflux depends on the activity of nigrostriatal dopamine neurones

P. Moleman & J. Bruinvels

Department of Pharmacology, Faculty of Medicine,
Erasmus University, PO Box 1738, Rotterdam, The Netherlands

It is clear that morphine markedly increases the turnover of dopamine (DA) in the rat striatum[1–3], although the nature of this increase remains obscure. The early suggestion, that morphine may block postsynaptic DA receptors in a similar manner to haloperidol[4], has since been refuted[5,6]. The effect of morphine on dopaminergic neurones seems to be presynaptic, but whether it stimulates[7,8] or inhibits[2,5] the functional activity of the dopaminergic neurones is unclear. We recently reported that morphine seems to have no effect on the functional activity of the nigrostriatal dopaminergic neurones, using the decline in DA after administration of α-methyl-*p*-tyrosine (αMT) as a measure of DA efflux[9,10]. However, the substantia nigra and striatum are extremely rich in opiate receptors[11,12], and a considerable number of the striatal opiate receptors seem to be localised on dopaminergic nerve endings[13]; it would therefore be rather surprising if stimulation of these receptors by morphine did not alter the functional activity of the nigrostriatal dopaminergic neurones. To investigate this apparent discrepancy, we analysed the effect of morphine on DA efflux in conditions of altered impulse flow in the nigrostriatal dopaminergic neurones. The present results show that the administration of morphine results in increased efflux of DA from neurones with a lowered firing rate, but not from neurones with a normal or increased firing rate. Thus, morphine may be a modulator of the nigrostriatal dopaminergic neurones in that it counteracts decreasing dopaminergic activity, but has no effect when the activity is normal or increased.

Male albino Wistar rats were treated with αMT methylester.HCl (254 mg per kg, intraperitoneally (i.p.)) half an hour before further treatment, which was aimed at altering the activity of the nigrostriatal neurones. These various treatments consisted of unilateral electrolytic lesion of the substantia nigra, apomorphine or haloperidol administration. Morphine (20 mg per kg, i.p.) was administered 17 min after lesion or together with apomorphine or haloperidol. Two hours after morphine administration the rats were decapitated and the striata were dissected out as described by Gispen *et al.*[14]; DA was assayed fluorimetrically as reported earlier[10].

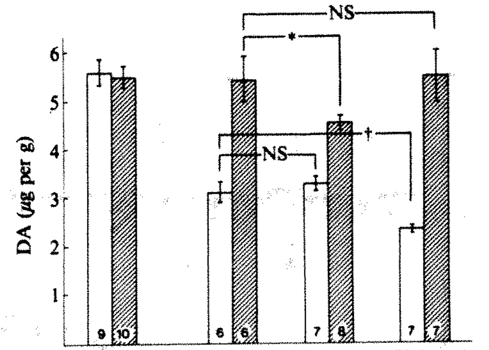

Fig. 1 Effect of morphine and haloperidol on the loss of striatal dopamine after αMT treatment in rats with unilateral lesion of the mesencephalic dopaminergic cell bodies. Male albino Wistar rats weighing 195–205 g were treated according to the time schedule given below. Unilateral electrolytic lesions of the right mesencephalic dopaminergic cell bodies were made stereotaxically by means of an iron electrode, 1 mm thick, coated with epoxy varnish except for 0.5 mm at the tip. A current of 2 mA was passed through the electrode for 3 min. The coordinates of the electrode were 6.6 mm caudal, 2.0 mm lateral and 7.7 mm ventral from the bregma. Midbrains were removed at the end of the experiment, fixed in formalin and examined histologically for electrode placements. All rats were injected with αMT 25 min before the lesion was made. Seventeen minutes after lesioning, the control rats were decapitated, while the other rats received morphine, haloperidol or saline injection. Most animals, at the moment of drug injection, had recovered from the light ether anaesthesia applied before lesioning. Rats were decapitated 2 h after injection of morphine, haloperidol or saline. Striata were dissected out according to Gispen *et al.*[14] and DA was extracted and assayed as reported elsewhere[10].

Time schedule of treatments (min)

Treatment group	0	25	42	162
Control	αMT	Lesion	Decapitation	–
Saline	αMT	Lesion	Saline	Decapitation
Morphine	αMT	Lesion	Morphine	Decapitation
Haloperidol	αMT	Lesion	Haloperidol	Decapitation

αMT: 254 mg per kg; morphine: 20 mg per kg; haloperidol: 1 mg per kg. All injections were made i.p. in a volume of 5 ml per kg. Drugs were dissolved in 0.9% NaCl. Statistics: Duncan's new multiple range test[22]. Values represent mean ± s.e.m.; number of observations are indicated at the base of the columns. Mean wet tissue weight: 46.1 mg ± 5.3. □, Control; ▨, lesion. * $P < 0.05$; † $P < 0.01$; NS, not significant.

Figure 1 shows that lesion of the mesencephalic dopaminergic cell bodies entirely prevented the loss of DA from the ipsilateral striatum. Injection of morphine resulted in loss of DA on the lesioned side but not on the side contralateral to the lesion, when compared with saline-treated rats. Haloperidol injection, on the other hand, resulted in increased loss of DA on the unlesioned side, but was without effect on the lesioned side. Thus, morphine seems to produce an efflux of DA from inactive neurones in the striatum but not from normally firing neurones. As the opposite is true for haloperidol, we conclude that the observed effect of morphine is not mediated by blockade of the DA receptors, which are blocked by haloperidol. In the nucleus accumbens similar effects of morphine and haloperidol were observed (unpublished results).

We then evaluated the effect of morphine on the loss of DA in rats pretreated with apomorphine or haloperidol. Apomorphine reduces and haloperidol increases the firing rate of the nigral

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DA neurones[15]. Treatment of the rats with apomorphine alone resulted in a reduction of ~50% in the loss of DA. In these apomorphine-treated rats, as in lesioned rats, administration of morphine resulted in increased DA loss. When the loss of DA was increased, however, by the administration of haloperidol, morphine had no effect (Table 1). Therefore, morphine administration apparently results in the efflux of DA in the striatum as a function of the impulse flow.

Table 2 shows that the effect of morphine was most pronounced when the impulse flow was entirely abolished, that it was less pronounced, but still significant, when the impulse flow was reduced by 50%, and that it was virtually absent at normal or increased impulse flow.

The present experiments allow conclusions to be drawn on the effect of morphine on DA release, if one assumes that the loss of DA after αMT treatment reflects DA release. During the first 30 min after αMT administration DA biosynthesis is not completely inhibited[9] and DA-releasing metabolites of αMT may be formed[16]. This time period is not suitable for studying DA release, therefore, but between 0.5 and 2.5 h after αMT administration the loss of DA seems to be a reliable parameter for functional DA release[17]. However, drug-induced alterations in the loss of DA could result from intraneuronal events apart from functional DA release. It has been suggested that morphine increases the intraneuronal metabolism of DA by displacing DA from storage sites to sites of metabolism[2]. However, it is unlikely that the present results could be explained by such a mechanism. Although morphine administration to apomorphine-treated rats resulted in increased loss of DA (Table 1), we have shown that in the same experimental conditions morphine has no effect on the concentrations of the DA metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (unpublished results). Additionally, DiChiara *et al.*[18] have shown that in mice not pretreated with αMT, the rise in DOPAC concentrations observed after morphine administration is entirely abolished in apomorphine-treated animals.

Table 1 Effect of morphine after αMT treatment on the loss of dopamine in the striatum of rats simultaneously treated with apomorphine or haloperidol

a

	DA* (μg per g)		% DA†	
	Saline	Apomorphine	Saline	Apomorphine
αMT+ saline	2.1 ± 0.10 (5)	3.1 ± 0.14 (7)‡	46 ± 2.2	69 ± 3.1
αMT+ morphine	1.9 ± 0.09 (6)	2.6 ± 0.07 (8)‡	41 ± 2.0	57 ± 1.6
P	NS	<0.005		

b

	DA* (μg per g)		% DA†	
	Saline	Haloperidol	Saline	Haloperidol
αMT+ saline	3.0 ± 0.08 (7)	1.8 ± 0.09 (8)‡	45 ± 1.2	27 ± 1.4
αMT+ morphine	2.8 ± 0.14 (8)	1.8 ± 0.08 (7)‡	42 ± 2.1	27 ± 1.2
P	NS	NS		

c Time-schedule of treatments (min)

0	30	90	150
αMT	Saline	Saline/—	Decapitation
αMT	Morphine	Saline/—	Decapitation
αMT	Apomorphine	Apomorphine	Decapitation
αMT	Apomorphine + morphine	Apomorphine	Decapitation
αMT	Haloperidol	—	Decapitation
αMT	Haloperidol + morphine	—	Decapitation

Male Wistar rats weighing 100–135 g were treated according to the time schedule given in *c*. Separate experiments were carried out with apomorphine (*a*), 4 mg per kg as HCl Salt, and haloperidol (*b*), 3 mg per kg. All drugs were dissolved in 0.9% NaCl. In the apomorphine experiment 0.2 mg ml^{-1} ascorbic acid was added to this. Stastistics: Student's *t*-test. Striata of one rat weighed 105 ± 8.3 mg ($n = 29$) in *a* and 101 ± 8.4 mg ($n = 34$) in *b* (mean ± s.d.). For other experimental details see Fig. 1 legend.

* Mean dopamine concentrations ± s.e.m., number of experiments in parentheses.

† Values represent mean dopamine concentrations ± s.e.m., expressed as % of control values in untreated rats. Dopamine concentrations in untreated rats (100%): *a*, 4.51 ± 0.17 μg per g ($n = 3$); *b*, 6.61 ± 0.25 μg per g ($n = 4$).

‡ Different from the appropriate saline group ($P < 0.001$).

Table 2 Effect of morphine on dopamine efflux in the striatum as a function of the activity of striatal dopaminergic neurones

Treatment to alter dopaminergic activity	Rate of dopamine loss Saline	Rate of dopamine loss Morphine	Effect of morphine (morphine – saline)
Lesion	4 ± 14%	33 ± 9%	+29%*
Apomorphine	48 ± 10%	68 ± 7%	+20%*
None	100 ± 9%	105 ± 11%	+5%
Haloperidol	162 ± 10%	162 ± 9%	0%

Rate constants of dopamine loss were calculated from the data presented in Fig. 1 and Table 1. The rate constants obtained from rats not treated to alter dopaminergic activity and not treated with morphine was set at 100%. The respective 100% values were: lesion experiment: $k = 0.292 \pm 0.0394\ h^{-1}$; apomorphine experiment: $k = 0.310 \pm 0.0291\ h^{-1}$; haloperidol experiment: $k = 0.323 \pm 0.0184\ h^{-1}$. The rate of dopamine loss in the experimental groups was calculated as a percentage of the above control values.

* Significant difference, see Fig. 1 and Table 1.

We conclude, therefore, from the present experiments, that administration of morphine results in increased dopamine release in the rat striatum, when the activity of the nigrostriatal neurones is decreased. As the administration of morphine resulted in release of dopamine in the striatum after lesion of mesencephalic cell bodies (Fig. 1), this effect does not seem to be mediated by the substantia nigra. It seems more likely, therefore, that morphine exerts its releasing effects by a direct action on the dopaminergic axons, perhaps by means of presynaptic opiate receptors on striatal dopaminergic neurones[13]. Whether the reported stimulation of dopaminergic neurotransmission by morphine via the substantia nigra[8,19] has an underlying mechanism similar to the effect reported here, remains to be established. The similarity in the effects of morphine and endorphins on striatal dopaminergic systems[20,21] suggests that one function of the endogenous opioid systems may be to counteract decreases in the activity of the nigrostriatal dopaminergic system.

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Correlation between benzodiazepine receptor occupation and anticonvulsant effects of diazepam

Steven M. Paul

Clinical Psychobiology Branch, National Institute of Mental Health,
National Institutes of Health, Bethesda, Maryland 20205

Peter J. Syapin

The Grant Neuroscience Laboratories, Department of Neurology,
School of Medicine, University of Southern California, Los Angeles,
California 90033

Beth A. Paugh, Victoria Moncada & Phil Skolnick

Laboratory of Bioorganic Chemistry, National Institute of Arthritis,
Metabolism and Digestive Diseases, National Institutes of Health,
Bethesda, Maryland 20205

The benzodiazepines are potent anticonvulsants for a wide variety of experimental and clinical seizure disorders[1,2]. The demonstration of saturable, high-affinity and stereospecific binding sites for the benzodiazepines in the mammalian central nervous system suggests the presence of pharmacological receptors mediating the anticonvulsant properties of these compounds[3,4]. The good correlation between the anticonvulsant potencies of a series of benzodiazepines and their ability to inhibit ^{3}H-diazepam binding *in vitro* further supports this hypothesis[3,4], but evidence for a direct interaction between benzodiazepines and their receptors, and a subsequent inhibition of seizure activity (or elevation of seizure threshold) is lacking[5]. Recent reports from our laboratory[6] and others[7] have demonstrated the feasibility of labelling benzodiazepine receptors *in vivo* following parental administration of tritiated benzodiazepine. This technique permits one to study the relationship between the anticonvulsant activity of the benzodiazepines *in vivo* and the number of 'drug-occupied' receptors *in vitro*. We now report that there is an excellent correlation between benzodiazepine receptor occupancy by diazepam and protection against pentylenetetrazol-induced seizures. Furthermore, these results demonstrate that only a small fraction of benzodiazepine receptors need be occupied to produce a complete anticonvulsant effect.

As previous studies have shown that the anticonvulsant activity of a single dose of diazepam decreases with time[5], groups of 20 male –C3H/HEN mice (16–21 g) were injected with diazepam (4 mg per kg, intraperitioneally (i.p.), Hoffman-LaRoche) and the number of 'drug-occupied' benzodiazepine receptors was determined simultaneously with its anticonvulsant effects during a 72-h period. At each time point examined, half of the mice in each group were killed and both the apparent number of benzodiazepine receptors occupied by diazepam and the total concentration of benzodiazepine in forebrain[8] were determined. The remaining mice were challenged with pentylenetetrazol (80 mg per i.p., K and K Laboratories) to determine the per cent protected against seizures. Pentylenetetrazol-treated mice were counted as having had a seizure only if generalised tonic–clonic movements were observed within 15 min of injection. This dose of pentylenetetrazol produced seizures in >95% of vehicle-treated control mice.

In confirmation of previous reports[5], the number of diazepam-treated mice having seizures in each group increased as a function of time following drug administration. Figure 1 shows the duration of the anticonvulsant effect following a single dose of diazepam. Greater than 90% of the mice were protected against seizures during the first 12 h after diazepam administration, and a significant protection was evident for as long as 48 h following treatment. However, at 60 and 72 h post-injection, all the mice exhibited seizure activity when challenged with pentylenetetrazol (Fig. 1 and unpublished observations).

The concentration of pharmacologically active 'non-membrane'-bound benzodiazepine in the brain decreased rapidly and was undetectable 24 h after diazepam administration, despite a significant anticonvulsant effect for an additional 24 h (Fig. 1). In contrast to the brain concentration of 'free' benzodiazepine, the number of benzodiazepine receptors occupied by diazepam (or its pharmacologically active metabolite, desmethyldiazepam) decreased slowly over time (Fig. 1). An excellent correlation ($r = 0.98$, $P<0.01$) was observed between the number of drug-occupied receptors and protection against pentylenetetrazol-induced seizures (Fig. 1, inset). Occupation of only a small fraction (<30%) of the total number of forebrain benzodiazepine receptors was sufficient to elicit a full anticonvulsant effect.

To distinguish between drug-occupied receptors and receptor desensitisation which could occur due to prior drug–receptor interaction, it was necessary to demonstrate the presence of receptor-bound benzodiazepine in the diazepam-treated mice. As incubation of the benzodiazepine–receptor complex at 37 °C results in a rapid dissociation of drug from receptor[9], crude synaptosomal membranes from diazepam-treated and vehicle-treated mice were incubated at 37 °C for 60 min followed by centrifugation at room temperature (12,000*g* for 15 min). The

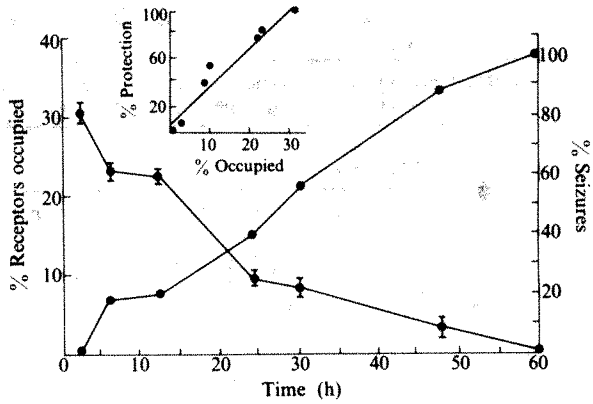

Fig. 1 Correlation between receptor occupation and anticonvulsant activity of diazepam. Mice were injected at time zero with diazepam (4 mg per kg, i.p.) and the number of drug-occupied benzodiazepine receptors was correlated with its anticonvulsant activity over the next 60 h. To determine the number of drug-occupied benzodiazepine receptors, mice were killed by decapitation and the crude synaptosomal fraction of forebrain (whole brain minus cerebellum and brainstem) was prepared by differential centrifugation[3]. The final synaptosomal pellet (P_2) was resuspended in 40 volumes of ice-cold Tris-HCl buffer (0.05 M, *p*H 7.4) to a final protein concentration of ~1 mg ml^{-1}. The corresponding supernatant (S_2) was frozen and stored at −20 °C for determining the free (unbound) concentration of benzodiazepine in brain[8]. Benzodiazepine receptors were measured in the resuspended P_2 fraction by modification of previously described methods[3]. The per cent of receptors occupied by benzodiazepine in synaptosomal membranes was determined by the formula: % occupied $= [(R_c - R_d)/B_{max}]100$, where $R_c = {}^3$H-diazepam bound in vehicle-treated mice, $R_d = {}^3$H-diazepam bound in diazepam-treated mice and B_{max} = maximum number of binding sites in vehicle-treated mice. The maximum number of binding sites in synaptosomal membranes from vehicle-treated mice was estimated by Scatchard analysis to be 1,213 fmol ^{3}H-diazepam bound per mg protein. R_c and R_d were determined in the presence of 4.4 nM ^{3}H-diazepam. At this concentration of ^{3}H-diazepam, $R_c = 653 \pm 10$ fmol ^{3}H-diazepam bound per mg protein ($n = 18$). The concentration of free or non-membrane-bound benzodiazepine was measured in the brain supernatants (S_2) using a radioreceptor assay[8].

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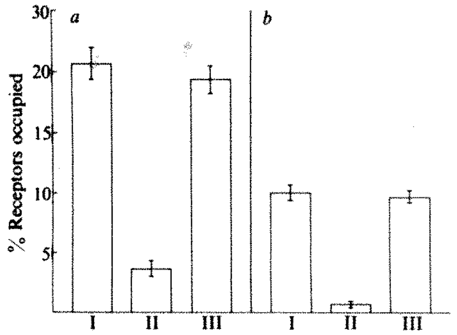

Fig. 2 Effects of *in vitro* incubation on receptor occupancy by benzodiazepines. Mice were injected with diazepam (4 mg per kg, i.p.) and synaptosomal membranes prepared 12 (*a*) or 24 (*b*) h later. Drug-occupied benzodiazepine receptors were determined as described in Fig. 1 legend. Group 1: % receptor(s) occupied by benzodiazepines. Group II: % receptors occupied following incubation of membranes from group I (37 °C for 60 min), centrifugation (12,000*g* for 15 min) and reconstitution with supernatant from vehicle-treated mice. Group III: % receptor(s) occupied in membranes of vehicle-treated mice reconstituted with supernatant obtained from group II (*n* = 6 mice per group).

resulting supernatants were carefully decanted and the supernatants from the diazepam-treated animals were reconstituted with synaptosomal membranes from the vehicle-treated animals; membranes from vehicle-treated mice were reconstituted with the supernatants from diazepam-treated animals. The reconstituted membranes from both groups were incubated for 30 min at 0–4 °C before incubation with ^{3}H-diazepam.

Incubation (37 °C for 60 min) of crude synaptosomal membranes from mice treated with diazepam 12 h (Fig. 2*a*) and 24 h (Fig. 2*b*) earlier resulted in a marked decrease in the number of drug-occupied receptors. This decrease seems to be the result of a temperature-induced dissociation of benzodiazepine from the receptor, as exchanging supernatants from the diazepam-treated and vehicle-treated animals results in the detection of 'occupied receptor' in membranes from vehicle-treated mice. Furthermore, the decrease in the number of drug-occupied receptors in incubated diazepam-treated membranes is directly proportional to the increase observed in the control membranes following incubation with the 'diazepam-treated' supernatant (Fig. 2*a*, *b*). The presence of pharmacologically active benzodiazepine in the supernatant (S_2) from the incubated diazepam-treated membranes was confirmed using a radioreceptor assay[8].

These results demonstrate a close association between the number of drug-occupied benzodiazepine receptors and the anticonvulsant activity of diazepam. In contrast, the total concentration of 'free' benzodiazepine in brain is poorly correlated with its anticonvulsant effects. Only a small fraction (25–30%) of the total number of benzodiazepine receptors need be occupied to manifest a complete anticonvulsant effect. This observation could indicate the presence of 'spare receptors' similar to that reported for various peripheral hormonal and neurotransmitter systems[10]. Alternatively, a specific population of benzodiazepine receptors may be involved in the anticonvulsant effects of diazepam, and the correlation obtained reflects preferential binding of diazepam to these receptors[11]. In addition to providing evidence for the importance of the benzodiazepine receptor in mediating the anticonvulsant effects of the benzodiazepines, these findings represent the first direct correlation between the occupation of a central receptor site and the clinical effects of a drug. Furthermore, they suggest that the benzodiazepine receptor and its presumed endogeneous ligand(s)[12] may normally be involved in preventing the discharge or spread of seizure activity within the CNS.

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Glycyrrhizic acid inhibits virus growth and inactivates virus particles

Raffaello Pompei, Ornella Flore, Maria Antonietta Marccialis, Alessandra Pani & Bernardo Loddo

Institute of Microbiology II, University of Cagliari, Italy

Screening investigations in antiviral action of plant extracts have revealed that a component of *Glycyrrhiza glabra* roots, found to be glycyrrhizic acid, is active against viruses. We report here that this drug inhibits growth and cytopathology of several unrelated DNA and RNA viruses, while not affecting cell activity and ability to replicate. In addition, glycyrrhizic acid inactivates herpes simplex virus particles irreversibly.

The effects of glycyrrhizic acid (ammonium salt, Fluka, Buchs) on growth of vaccinia, herpes simplex type 1, Newcastle disease, vesicular stomatitis (Indiana type) and polio type 1 viruses (all provided by NIH) in cultures of human aneuploid HEp2 cells (ATCC, Rockville) where studied. Twenty-four-hour-old cell monolayers (10^7 cells per sample) were infected with 5 infectious units per cell of each virus at 20 °C for 1 h, washed 3 times in Hank's balanced saline solution (BSS) and incubated at 37 °C for 18 h in Eagle's minimum essential medium (MEM) supplemented with 2% calf serum (*p*H 7.4). Infectious virus yield was determined by the Dulbecco and Vogt technique[1], slightly modified for vaccinia and herpes simplex type 1 viruses[2]. Cytopathic effects were evidenced by light microscope observation of Giemsa-stained cells and by measuring spectrophotometrically at 530 nm the amount of neutral red incorporated by cell cultures (100 μg ml^{-1}, 1-h pulses in a drug-free medium) after solubilisation in 1% sodium deoxycholate in BSS (*p*H 7.4)[3].

As shown in Table 1, addition of 8 mM glycyrrhizic acid to infected cell cultures soon after incubation at 37 °C completely inhibits both growth and cytopathic effects of vaccinia, herpes simplex type 1, Newcastle disease and vesicular stomatitis viruses. Protection of cell cultures from viral damage is so effective that, in spite of the total infection produced by the virus input used, differences can hardly be found at microscopic observation levels between drug-treated infected cells and uninfected controls. Later treatment (3 h post-infection) of cell cultures with 8 mM glycyrrhizic acid still suppresses virus replication and stops the progress of the cytopathic effect. Glycyrrhizic acid is also active on virus growth at 4 mM and at 2 mM, but not at 1 mM (data not shown). On the other hand, glycyrrhizic acid is without effect on poliovirus type 1.

Glycyrrhizic acid treatments that were inhibitory to virus growth were tolerated by cell cultures.

Uninfected cells incubated at 37 °C for 36 h in the presence of 8 mM glycyrrhizic acid remained unaltered both morphologically (evaluated as above) and in their ability to incorporate

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Table 1 Effect of glycyrrhizic acid on virus growth and cytopathology

Virus strain input (5 i.u. per cell)	Virus growth and cell damage after 18 h at 37 °C in: Drug-free medium			Medium supplemented with 8 mM glycyrrhizic acid: Immediately after infection			3 h postinfection		
	i.u.	CPE	NR	i.u.	CPE	NR	i.u.	CPE	NR
Vaccinia	6.8×10^7	+++	45	2×10^4	−	99	3×10^4	+−−	86
Herpes simplex type 1	3.4×10^7	+++	32	$<10^4$	−	97	$<10^4$	+−−	84
Newcastle disease	1.3×10^7	+++	<5	6×10^4	−	102	9×10^4	+−−	82
Vesicular stomatitis	1.2×10^8	+++	<5	6×10^4	−	96	1.3×10^5	+−−	78
Polio type 1	7×10^7	+++	<5	4.6×10^7	+++	<5	5.2×10^7	+++	<5

i.u., Infectious units; CPE, cytopathic effect; +++, complete CPE, −, absence of CPE, evaluated microscopically; NR, neutral red uptake in % of untreated uninfected controls.

Table 2 Effects of glycyrrhizic acid on uninfected cell cultures

Glycyrrhizic acid in medium	^{14}C amino acid uptake* (c.p.m.)	No. of cells per ml of medium†
—	76.705 (74.534–80.212)	7.1×10^5 ($6.2\times10^5-7.9\times10^5$)
8 mM	73.952 (71.528–77.334)	5.2×10^5 ($4.2\times10^5-6.4\times10^5$)

Figures are mean values of six determinations. The range of values is given in parentheses.

* ^{14}C amino acid uptake (0.3 μCi ml^{-1}, 1-h pulses) was measured after 35 h at 37°C in Eagle's MEM 2% serum.

† Initial inoculum, 2.5×10^5 cells ml^{-1}. Count made after 36 h at 37° C in Eagle's MEM 7% serum.

amino acids in acid-insoluble form[4], as determined by 1-h pulses of ^{14}C-protein hydrolysate (40 mCi mmol^{-1}, 0.3 μCi ml^{-1}, Amersham) (Table 2). Addition of 8 mM glycyrrhizic acid to 4-h-old cell cultures incubated in Eagle's MEM 7% calf serum had virtually no effect on cell growth. Thoma chamber counts of cells suspended by trypsin (0.25%, Difco) revealed a 28% decrease in cell growth in the respect of untreated controls, after 36 h at 37 °C.

Taking into account that in antiviral tests glycyrrhizic acid was allowed to act on infected cultures for 18 h (the time required for growth of all viruses in untreated controls), these data indicate that the antiviral effect of this drug is not mediated by damaging the cells.

Apart from inhibiting growth of several viruses, glycyrrhizic acid also produces irreversible inactivation of herpes simplex virus type 1. Suspensions of this virus suffer a loss of infectivity of 10^5 when incubated at 37 °C with 8 mM glycyrrhizic acid for only 15 min. Similar treatments do not inactivate the other viruses (at least irreversibly), even if prolonged for 3h.

Table 3 Inactivating effect of glycyrrhizic acid on virus particles

Virus strain	Incubation mixture at 37°C	i.u. Evidenced*
Vaccinia	in MEM	5.3×10^7
	in 8 mM GA	5.6×10^7
Herpes simplex type 1	in MEM	1.2×10^7
	in 8 mM GA	1.3×10^2
Newcastle disease	in MEM	3.9×10^7
	in 8 mM GA	4.2×10^7
Vesicular stomatitis	in MEM	4.3×10^7
	in 8 mM GA	3.1×10^7
Polio type 1	in MEM	6.1×10^7
	in 8 mM GA	3.9×10^7

* By the Dulbecco and Vogt method[1] in incubation mixtures diluted in drug-free MEM.

5×10^7 i.u. of each virus was incubated for 180 min, except in the case of herpes simplex type I in 8 mM GA, which was for 15 min. GA, glycyrrhizic acid.

Data from current research had led us to hypothesise that glycyrrhizic acid interacts with sensitive virus proteins both at the virionic stage and later on, when these proteins are synthesised in host cells. This is, however, a working hypothesis that has yet to be verified experimentally.

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Ca^{2+}-induced fusion of phospholipid vesicles monitored by mixing of aqueous contents

Jan Wilschut & Demetrios Papahadjopoulos

Cancer Research Institute, University of California, School of Medicine, San Francisco, California 94143

Ca^{2+} has a central role in various cellular phenomena involving membrane fusion[1–3]. However, little is known about the mechanisms involved. Model membrane systems such as phospholipid vesicles have been used extensively to study the mechanism of membrane fusion at the molecular level[4]. For example, phosphatidylserine (PS) vesicles have been shown to undergo massive aggregation and structural rearrangements on addition of Ca^{2+}, with eventual formation of large cochleate structures[5–8]. Although these structures do not retain appreciable internal volume, their formation has been proposed to result from fusion of the initial vesicles[5–8]. The significance of the PS–Ca^{2+} system as a model for biological membrane fusion has been questioned recently by Ginsberg[9]. Based on the observation that divalent cations induce the release of contents from PS vesicles but fail to bring about the uptake of a marker from the medium, he proposes that the vesicles are ruptured completely during interaction with divalent cations and reassemble subsequently to form large non-vesicular structures. The present study demonstrates that the question raised by Ginsberg[9] is not particularly relevant to the phenomenon concerned, and that his experimental observations do not allow the exclusive conclusion that Ca^{2+} induces lysis of PS vesicles rather than fusion.

With respect to the question of 'lysis or fusion', in general, membrane fusion is not necessarily a completely non-leaky process. Therefore, release of vesicle contents induced by

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divalent cations does not preclude a fusion event being involved. However, the question of whether the release of contents depends on mutual interaction between the vesicles is crucial. Our recent observation that Ca^{2+}-induced leakage of PS vesicle contents is approximately second-order with respect to vesicle concentration[8] indicates that vesicle–vesicle interaction is a prerequisite for the observed release and suggests that leakage from separate PS vesicles does not occur. The observation by Ginsberg that Ca^{2+} fails to bring about the uptake or entrapment of markers from the external medium into the vesicles[9] can be completely reconciled with earlier results[5,6] regarding the formation of large cochleate structures from PS vesicles by interaction with Ca^{2+}. These cochleates have been shown to result from the eventual collapse of the vesicles and, therefore, do not retain any encapsulated volume[7,8]. This is indicated by the very short lamellar distance of 53 Å in the PS–Ca^{2+} membrane system, determined by X-ray diffraction[7], and the absence of any appreciable amounts of water between the bilayers in the final complex[8]. Consequently, it is highly unlikely that solutes from the medium would be taken up and retained in the structure during a 1-h incubation. However, the final formation of such a tightly packed PS–Ca^{2+} membrane complex does not necessarily lead to the conclusion that the sequence of events involved is one of lysis of separate vesicles with subsequent reassembly into non-vesicular structures, as proposed by Ginsberg[9]. As the author admits, fusion might occur during the initial stages of the interaction between vesicles. However, the design of his experiments did not permit investigation of the early events in this process.

To be able to follow the kinetics of vesicle fusion phenomena, we have developed an assay which meets the rigorous criterion of mixing of vesicle contents. The method is based on the interaction between terbium (Tb^{3+}) ions and dipicolinic acid (DPA)[10–12]. Formation of the $Tb(DPA)_3^{3-}$ complex produces a 10^4-fold enhancement of the Tb fluorescence[11,12]. The design of the assay involves encapsulation of $TbCl_3$ in one population of vesicles and DPA in another. $TbCl_3$ is encapsulated in the presence of a 10-fold molar excess of citrate as a weak chelator, which prevents interaction of Tb^{3+} with the negatively charged lipid. DPA is encapsulated at a 10-fold higher molar concentration than $TbCl_3$. On fusion of the two types of vesicle the Tb^{3+}–citrate complex is expected to dissociate in favour of the formation of the chelation complex between Tb^{3+} and dipicolinate, due to the very high affinity of the latter for Tb^{3+} (ref. 10). Release of vesicle contents is not registered as fusion because Ca^{2+} and EDTA, both present in the external medium, strongly interfere with the formation of the fluorescent $Tb(DPA)_3^{3-}$ complex through their interaction with DPA and Tb^{3+}, respectively. Introduction of 0.1 mM EDTA into a solution containing 1 μM $TbCl_3$, 10 μM citrate and 10 μM DPA in the buffer in which the fusion assay is done produces a 90% decrease of the fluorescence intensity. The additional presence of Ca^{2+} at a concentration of 1 mM or higher further reduces the fluorescence intensity to 1% of the maximal value. Moreover, during the fusion assay the quenching potencies of Ca^{2+} and EDTA will be even more pronounced, as the overall Tb, citrate and DPA concentrations are about seven-fold lower than those in the control experiment mentioned above. Consequently, only mixing of vesicle contents, separated from the outside medium, is registered as fusion. An additional advantage of this assay is that the formation of the $Tb(DPA)_3^{3-}$ complex is extremely fast and thus allows an accurate kinetic analysis of the initial events during vesicle-vesicle interactions. A more extensive account of the assay will be presented elsewhere.

Figure 1 shows the kinetics of mixing of vesicle contents induced by 1.5 mM Ca^{2+} in a 1:1 mixture of two PS vesicle populations containing Tb and DPA, incorporated in separate vesicles, as monitored by the increase of Tb fluorescence (solid line). The design of the fluorimeter permitted the simultaneous recording of the kinetics of vesicle aggregation, as measured by light scattering (dotted line). In addition, Fig. 1 shows the release of carboxyfluorescein (CF) from a similar vesicle preparation induced by the same Ca^{2+} concentration (dashed line). It is apparent from these results that Ca^{2+} induces a very rapid mixing of the vesicle contents, which occurs on the same time scale as the initial aggregation. To exclude the possibility that the observed increase in fluorescence resulted from the formation of $Tb(DPA)_3^{3-}$ complexes in close proximity but outside the vesicles, possibly due to release to locally high concentrations,

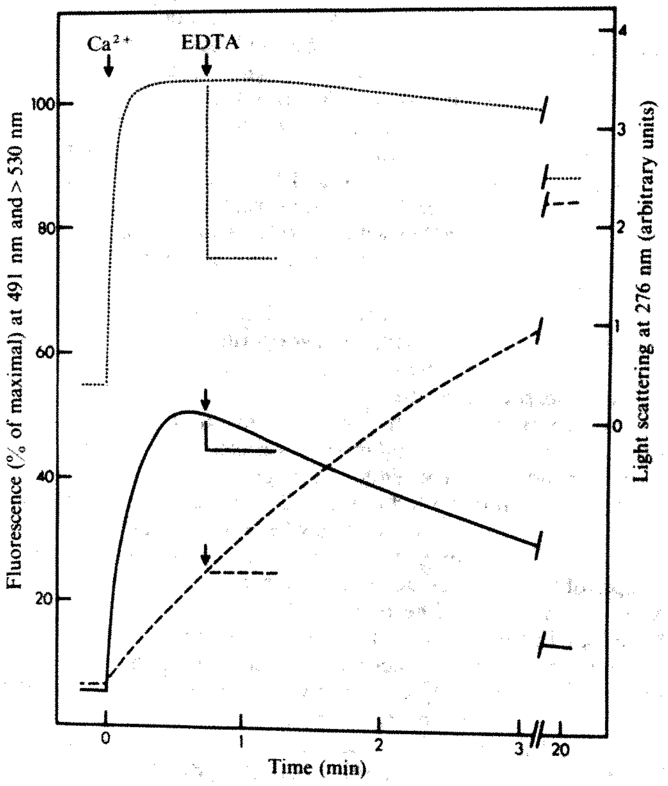

Fig. 1 Calcium-induced fusion of PS vesicles and release of vesicle contents. Unilamellar vesicles were prepared from bovine brain phosphatidylserine (PS) by sonication under nitrogen in a bath-type sonicator for 1 h at 20 °C (ref. 6). Lipid concentration during sonication was 10 μmol ml^{-1}. Thin-layer chromatographic analysis[17] of the lipid in vesicle preparations after sonication (with a load of 6 μg of lipid phosphorus per spot on the thin-layer plate) showed no detectable amounts of degradation products such as lyso-PS and fatty acids. Vesicles were prepared in (1) 10 mM $TbCl_3$/100 mM sodium citrate/2.0 mM L-histidine/2.0 mM N-Tris(hydroxymethyl)methyl-2-aminoethanesulphonic acid (TES), *p*H 7.4; (2) 100 mM sodium dipicolinate (DPA)/2.0 mM L-histidine/2.0 mM TES, *p*H 7.4 or (3) 100 mM carboxyfluorescein (CF)/2.0 mM L-histidine/2.0 mM TES, *p*H 7.4, and separated from non-encapsulated material by gel filtration on Sephadex G-75. Elution buffer, 100 mM NaCl/2.0 mM L-histidine/2.0 mM TES/1.0 mM EDTA, *p*H 7.4. Phospholipid phosphorus was determined as described in ref. 18. Carboxyfluorescein was purified as described in ref. 13. All chemicals were of the highest purity available[8]. Fusion experiments were carried out in quartz fluorescence cells containing 1.0 ml of 100 mM NaCl/2.0 mM L-histidine/2.0 mM TES/0.1 mM EDTA, *p*H 7.4, at a total lipid concentration of 100 μM. Tb- and DPA-containing vesicles were mixed in a 1:1 ratio, 50 μM each. The solution was stirred with a magnetic stirring bar and the temperature was maintained at 25 °C. $CaCl_2$ was injected as a 100 mM solution to a final concentration of 1.5 mM. In separate experiments EDTA was injected as a 100 mM solution, 45 s after the addition of $CaCl_2$, to a final concentration of 5.0 mM. Fluorescence was measured in an SLM-4000 instrument (SLM Instruments) with a 'T'-design, permitting simultaneous recording of 90° fluorescence and 90° light scattering. Excitation of Tb fluorescence was at 276 nm, emission was detected at 491 nm (solid line), and light scattering was measured using a Corning 7-54 bandpass filter (dotted line). Excitation of CF fluorescence was at 460 nm and emission was measured at >530 nm using a Corning 3-68 cut-off filter (dashed line). The value of 100% Tb fluorescence was determined in the presence of 20 μM DPA by releasing the contents from 50 μM Tb-containing vesicles (freed from EDTA on Sephadex G-75) with 0.5% (w/v) sodium cholate. Maximal CF fluorescence was determined in the presence of 0.1% (v/v) Triton X-100. Fluorescence intensity is presented as a percentage of the maximal values. The light scattering is expressed in arbitrary units; note that the curve is shifted on the vertical scale.

we added excess EDTA to the mixture to terminate the fusion reaction once the maximum level of fluorescence had been attained. As shown in Fig. 1, the fluorescence intensity could be maintained at a high level, indicating a physical separation of the fluorescent complex from the external EDTA-containing medium. Although release of vesicle contents, as monitored by increasing CF fluorescence[8,13], started immediately after Ca^{2+} addition, its rate was considerably lower than that of the Tb fluorescence increase. This indicates that fusion proceeds more rapidly than the release of vesicle contents. The Tb fluorescence reached its maximal level, corresponding to ~50% of the total amount of Tb present, within 45 s after the addition of Ca^{2+}. At that time, only 20–25% of the CF had been released. On addition of 2.0 mM Ca^{2+}, rather than 1.5 mM, the maximal level of Tb fluorescence approached 65% (not shown). These observations imply that multiple fusion events must occur, for otherwise, assuming no release at all, in a 1 : 1 mixture of the two types of vesicle only a maximum of 50% of the Tb can become associated with DPA. The eventual decrease of the Tb fluorescence (Fig. 1) is consistent with the release and dissociation of the $Tb(DPA)_3^{3-}$ complex in the medium. Addition of EDTA, 45 s after the addition of Ca^{2+} (Fig. 1) not only produces a constant high level of Tb fluorescence, but also stops CF release and induces a decrease in the light scattering to a level which is considerably higher than the level before Ca^{2+} addition, indicating increased vesicle size.

From the present results, we conclude that Ca^{2+} induces extensive fusion of PS unilamellar vesicles as monitored by mixing of aqueous contents. Leakage of vesicle contents, although remaining limited initially, does occur. Consequently, Ca^{2+} can slowly enter the vesicles where it can bind to the PS molecules of the interior monolayer, causing subsequent collapse of the fused vesicles and further release of contents. The assay system described here has made it possible to distinguish the initial fusion of PS vesicles from the release of vesicle contents. Studies with vesicles composed of various lipid mixtures are now in progress to investigate the possibility of producing vesicles which are capable of fusing but are more resistant to leakage than vesicles composed of pure PS. In any case, the present results and other recent reports[8,14–16] indicate that Ca^{2+}-induced vesicle fusion is quite relevant to biological membrane fusion phenomena, and could considerably enhance our understanding of the mechanism of such events at the molecular level.

$TbCl_3 \cdot 6H_2O$ was obtained from Alfa, dipicolinic acid from Sigma and carboxyfluorescein from Eastman Kodak. One of us (J.W.) is on leave from the University of Groningen, supported by the Netherlands Organisation for the Advancement of Pure Research (ZWO). This work was supported by grant GM 26369 from the NIH.

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Differences in A and B forms of monoamine oxidase revealed by limited proteolysis and peptide mapping

Richard M. Cawthon & Xandra O. Breakefield*

Department of Human Genetics, Yale University School of Medicine,
New Haven, Connecticut 06510

Monoamine oxidase (MAO, EC 1.4.3.4) is a mitochondrial outer membrane enzyme which deaminates amine transmitters in the nervous system and biogenic amines throughout the body. At least two types of MAO activity have been identified which differ in their specificities for substrates and sensitivities to inhibitors[1–3]. Type A preferentially deaminates 5-hydroxytryptamine and noradrenaline and is more sensitive to inhibition by clorgyline. Type B deaminates phenylethylamine and benzylamine and is more sensitive to inhibition by deprenyl. Tissues from several species express MAO A alone, MAO B alone, or both types of MAO. Furthermore, the two types of activity follow different developmental sequences in certain tissues. Models to explain the molecular and genetic regulation of this enzyme[4] raise two questions: (1) are there different enzyme molecules associated with A and B types of activity, and (2) if there is only one enzyme molecule, how do differences in the microenvironment of this enzyme within the outer mitochondrial membrane produce two types of activity? Attempts to separate and purify the different types of activity have involved solubilisation and lipid extraction procedures that alter the kinetics of the enzyme and differentially affect the A and B types of activity[5–8]. Immunological studies[9,10] have produced equivocal results. None of these observations has elucidated the molecular basis of the differences between the types of MAO activity. Here, we have used the technique of proteolytic digestion and peptide mapping to demonstrate that there are distinct enzyme molecules associated with the A and B types of MAO activity. This finding implies that the A and B forms of MAO may be coded for by separate gene loci and represent evolutionarily related proteins which have diverged in function.

Pargyline, an irreversible inhibitor of both A and B types of MAO activity[11–16], binds stoichiometrically to MAO, forming a stable adduct with the flavin residue[16]. This reaction is specific for MAO and does not occur with other purified flavoproteins[17]. Pargyline binding was used with limited proteolysis and one-dimensional peptide mapping, an effective technique for detecting differences and similarities in the amino acid sequences of proteins of similar molecular weights (MWs)[18–20]. The pattern of fragments obtained is highly reproducible and characteristic for a given substrate protein and proteolytic enzyme. As they are known to have similar MWs[14,15], the structure of the A and B forms of MAO in rat hepatoma cells was investigated by site-specific proteolysis in the presence of SDS, followed by electrophoresis in SDS-polyacrylamide gels to identify peptide fragments.

Following binding of ^3H-pargyline to crude mitochondrial preparations, proteins were fully solubilised and denatured by heating in high concentrations of SDS in reducing conditions (see Fig. 1 legend), and electrophoretically separated on SDS-polyacrylamide gels. Figure 1 shows ^3H-pargyline-labelled peptide fragments detected when radiolabelled MAO was isolated and exposed to *Staphylococcus aureus* V8 protease and further SDS-polyacrylamide gel electrophoresis. The peptide maps in each panel are chosen to illustrate all the fragments obtained when identical samples of ^3H-pargyline-labelled MAO were incubated with five concentrations of protease. The first three panels display proteolytic fragments of MAO from rat

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* To whom correspondence should be addressed.

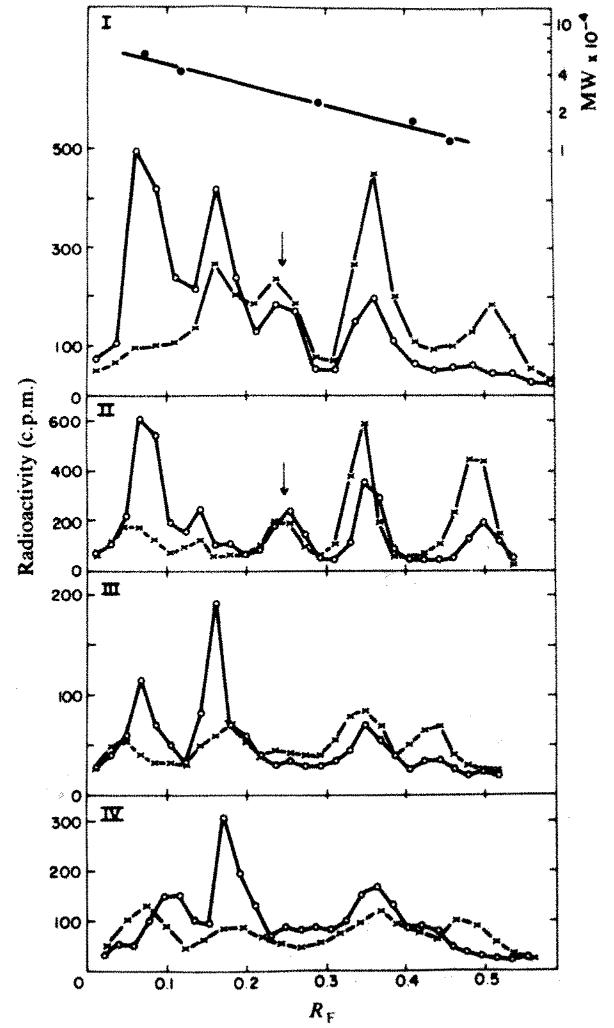

Fig. 1 Peptide maps of the A and B forms of MAO produced by limited proteolysis with *S. aureus* V8 protease and electrophoresis in 15% SDS-polyacrylamide gels. The migration relative to the tracer dye front (R_F) is plotted on the abscissa, and the radioactivity of each slice and the MW are plotted on the left and right ordinates, respectively. The conditions and procedures used to grow and subculture the cell lines used were as described previously[14], except that for subculturing, cells were resuspended in isotonic buffered saline containing no Ca^{2+} or Mg^{2+} and supplemented with 1 mM EDTA. Crude mitochondrial fractions were prepared from cultures collected at confluency as described elsewhere[14], and stored at −20 °C or used immediately. To label MAO, mitochondrial preparations (400 μg protein per sample) were incubated with 0.29 nmol [^{3}H-benzylmethylene]pargyline (6.86 Ci mmol^{-1}, NEN) in a final volume of 1.0 ml 50 mM Tris-HCl, *p*H 7.5, for 60 min at 37 °C in a shaking water bath. Preparations in panel II were preincubated with 0.1 μM clorgyline at 37 °C for 30 min before addition of ^{3}H-pargyline. Preparations in panel III were preincubated with 1.0 μM deprenyl at 37 °C for 15 min before addition of ^{3}H-pargyline. To ensure that the drugs did not interfere with the proteolysis of MAO, some mitochondrial preparations were incubated with clorgyline or deprenyl only after the ^{3}H-pargyline radiolabelling step. When all incubations were completed, the samples were centrifuged at 40,000*g* for 30 min at 4 °C. The radiolabelled mitochondrial pellets were resuspended in 160 μl of a loading solution containing 3% SDS, 0.15 M sucrose, 1% β-mercaptoethanol, 0.005% bromphenol blue, 3 mM EDTA and 0.0625 M Tris-HCl, *p*H 6.8, and proteins were solubilised by boiling for 5 min. Preparative polyacrylamide slab gel electrophoresis was carried out in a 7.5% acrylamide running gel (15 × 10 × 0.3 cm) with a 3% stacking gel (15 × 2 × 0.3 cm) both containing 0.1% SDS, using a modification[23] of the procedure of Maizel[24]. To locate the position of MAO in the preparative gel, a lane containing radiolabelled MAO and a lane containing standard MW marker proteins were sliced from the gel and stained and destained as described elsewhere[18]. The lane containing the mitochondrial sample was cut into 2-mm slices along a 2-cm section in the region of the 58,000 MW marker, catalase. The remainder of the preparative gel was stored, unstained, at −20 °C. The distribution of the radioactivity in the slices was determined quantitatively using a modification of the method of Horvitz[25] as recommended by NEN. Each slice was placed in a glass scintillation vial and 10 ml of a solution containing 5% Protosol and 7.5% Liquifluor (v/v, NEN) in toluene were added. Samples were incubated for 16 h at 37 °C and radioactivity was determined by liquid scintillation spectrometry. A single peak of radioactivity was observed in the sliced region, indicating the precise location of MAO. The remainder of the preparative gel was then thawed, stained and destained in <1 h[18], and cut to obtain nine gel slices, one 9 × 3 × 3-mm slice per lane, which contained the radiolabelled MAO. The slices, and the sample wells of the proteolytic gels, were treated using a modification[19] of the method of Cleveland *et al.*[18]. The proteolytic gels were prepared as described[18] except that they were cast 3 mm thick with sample wells 2.5 cm deep and 1.0 cm wide. Three gel slices containing radiolabelled MAO were stacked at the bottom of each sample well. Six identical samples of three slices each were routinely loaded onto each gel. A concentrated solution of *S. aureus* V8 protease (36-900-1, Miles), stored at −20 °C, was thawed, and serial dilutions were prepared and added to the sample wells in amounts of 0, 0.005, 0.025, 0.1, 0.5, and 2.5 μg per well. Standard MW marker proteins solubilised in loading solution (see above) were applied to the remaining sample well. The marker proteins used were catalase, 58,000 (Calbiochem); ovalbumin, 43,000; trypsin, 23,300; myoglobin, 17,200; cytochrome *c*, 12,400 (Sigma). Electrophoresis was carried out at a constant current of 45 mA, except for a 30-min period when current was turned off, after the tracer dye had neared the bottom of the stacking gel. Electrophoresis was continued until the bromphenol blue tracer dye in the marker lanes had moved ~10 cm into the running gel. The gels were then stained and destained in <1 h, as above. Each lane was cut into approximately 50 2-mm slices and the distribution of radioactivity in the slices was quantitated as described above. A plot of the mobilities of the marker proteins against the log of their MWs was used to estimate the MWs of the radiolabelled peptide fragments. Panels I, II and III show peptides from line MH_1C_1: I, mitochondria were labelled with ^{3}H-pargyline; II, mitochondria were incubated with clorgyline before ^{3}H-pargyline labelling; III, mitochondria were incubated with deprenyl before ^{3}H-pargyline labelling. Panel IV shows mitochondria from line C6, labelled with ^{3}H-pargyline. Each panel shows peptide patterns from two of the five concentrations of protease tested. These patterns were chosen because they included all the peptide fragments observed at all protease concentrations. Results shown are from one of two or three similar experiments. Control samples of MH_1C_1 incubated with clorgyline or deprenyl only after ^{3}H-pargyline labelling yielded peptide maps essentially identical to that in panel I. In control samples not treated with protease, all radioactivity above background levels was found in the leftmost peak, corresponding to the MW of intact MAO—57,000.

hepatoma line, MH_1C_1. Panel I shows peptide fragments derived when both the A and B forms of MAO were labelled. Panel II shows peptides exclusively from the B form, as binding of ^{3}H-pargyline to the active site of type A MAO was blocked by preincubation of the mitochondrial preparations with clorgyline. Panel III shows labelled peptides exclusively from the A form, as binding of ^{3}H-pargyline to the active site of type B MAO was blocked by preincubation with deprenyl. The ^{3}H-pargyline-labelled peptides displayed in panel IV resulted from limited proteolysis of MAO from rat glioma line C6. This cell line has only type A MAO activity[21] and was derived from a different strain of rats from the MH_1C_1 line.

A peptide fragment of approximately MW 29,000 is prominent in the maps shown in panels I and II, but is missing from the maps in panels III and IV. This fragment was present only when the B form of MAO was labelled. The other three fragments in the maps were common to both A and B forms of MAO. In preliminary studies the proteolytic enzyme papain was found to generate four ^{3}H-pargyline-labelled peptide bands (MW >10,000) which were common to both forms of MAO. Proteolysis by chymotrypsin produced two labelled bands common to A and B and one band unique to the A form (data not shown).

The flavin-containing polypeptides which underlie the A and B types of MAO activity in the rat are similar in MW[14,15]. Furthermore, radiolabelled MAO A and B from rat hepatoma and glioma cells have identical mobilities (M.R.C. Costa and X.O.B., unpublished) in a non-equilibrium *p*H gradient gel system[22], showing they also have a similar charge. Discrete regions in these polypeptides were analysed using site-specific proteases in denaturing and solubilising conditions for MAO. The different peptide maps obtained for MAO A and B indicate differences in the location of the target sites for the protease, or in their susceptibility to proteolysis. These differences could result from divergent amino acid sequences or differential post-translational modifications. Such molecular differences may determine the association of these polypeptides with other molecules, and the final position and conformation of the enzyme within the mitochondrial membrane. An important hypothesis that is ruled out by these data is that the differences underlying the A and B types of MAO activity are solely due to the membrane microenvironments of identical enzyme molecules.

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Note added in proof: Using SDS-polyacrylamide gel electrophoresis (SDS-PAGE), Callingham and Parkinson found that in rat tissues the pargyline binding polypeptides associated with type A and B MAO activities have MWs of 60,000 and 55,000, respectively (MAO Symposium, Midland, Michigan, 1979). We subsequently modified our SDS-PAGE technique and have now demonstrated a similar MW difference for these polypeptides in rat hepatoma cells, providing additional support for the conclusion that MAO A and B are distinct enzyme molecules.

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Structure of two spliced mRNAs from the transforming region of human subgroup C adenoviruses

Michel Perricaudet

Recombination et expression génétique (Institut National de la Santé et de la Recherche Médicale U163), Unité de Génie Génétique, Institut Pasteur, 28 rue du Dr. Roux, 750 15 Paris, France

Göran Akusjärvi, Anders Virtanen & Ulf Pettersson

Department of Microbiology, University of Uppsala, Box 581,
S-751 23 Uppsala, Sweden

The papova viruses and the human adenoviruses are widely used as a model system to study cell transformation *in vitro*. In subgroup C human adenoviruses, fragment *Hpa*I-E, which comprises as little as 4.5% of the adenovirus type 5 (ad5) DNA, is sufficient for transformation of rat embryo cells[1]. Analysis of messenger RNAs (mRNAs) from the transforming region of adenoviruses type 2 (ad2) has identified several spliced mRNA species[2–4]. Promoter mapping studies indicate that the leftmost early region contains two separate transcription units, E1A and E1B (ref. 5) (Fig. 1*a*). Region E1A is approximately equivalent *Hpa*I-E. The complete nucleotide sequence of the *Hpa*I-E fragment of ad5 was recently reported[6]. However, the spliced nature of early adenovirus mRNAs prevents a prediction of the amino acid sequence of the corresponding polypeptides directly from the DNA sequence. To study the structure of early ad2 mRNAs at the nucleotide level, we have used molecular cloning procedures to amplify the appropriate mRNA sequences. In this report, clones corresponding to the 12S and 13S mRNA from region E1A (Fig. 1*c*) have been isolated and characterised by hybridisation and sequence analysis. Our results enable us to predict the primary sequence of two related polypeptides from region E1A of human subgroup C adenoviruses.

To clone early ad2 mRNAs, double-stranded complementary DNA (cDNA) copies were inserted into the *Pst*I site of plasmid pBR322 after dG/dC tailing with terminal transferase. Clones containing ad2-specific sequences were identified by hybridisation[7], using ad2 DNA or specific fragments of ad2 DNA as probes. In this way three clones were identified which contain sequences from region E1A of the ad2 genome. These clones are designated 147, 131 and 133. Plasmid DNA was extracted from these clones, labelled *in vitro* by nick translation and hybridised to *Sma*I fragments of ad2 DNA. Plasmid DNA from clones 131 and 133 hybridised to both fragments *Sma*I-J and *Sma*I-E (map coordinates 0–2.9 and 2.9–10.7) whereas clone 147 hybridised exclusively to fragment *Sma*I-E (Fig. 2).

The clones were further analysed by restriction enzyme cleavage using endonucleases *Hpa*I, *Xba*I, *Sma*I and *Pst*I. Because the cDNAs were inserted into the *Pst*I site of plasmid pBR322 by dG/dC tailing, endonuclease *Pst*I will excise the cDNA insert. Clone 147 contained a 440-nucleotide-long insert which lacked the cleavage site for *Sma*I whereas *Hpa*I cleavage resulted in two fragments, 130 and 310 base pairs long. From these results we conclude that clone 147 contains sequences corresponding to the 3′-end of a mRNA from region E1A (see Fig. 1*a–c*).

Clone 133 contained a cDNA insert 840 base pairs long that lacked a cleavage site for *Sma*I, whereas *Xba*I cleavage gave two fragments 620 and 220 base pairs long. These results together with the hybridisation results indicate that clone 133 corresponds to the 12S mRNA of region E1A (Fig. 1*c*).

The cDNA insert in clone 131 was approximately 650 base pairs long, and was cleaved precisely in the middle by *Sma*I. Cleavage with *Xba*I resulted in two fragments, 510 and 140 base pairs long. The presence of a cleavage site for *Sma*I in the cDNA insert suggests that clone 131 is related to the 13S mRNA of region E1A although it is not a complete copy of this mRNA.

To study the structure of the cloned mRNAs at the nucleotide level, sequence analysis according to the Maxam and Gilbert[8] procedure was carried out. The strategy for our sequencing approach is shown in Fig. 1*b* and *d*, which also outlines the results. The proposed sequence for the 12S and 13S mRNAs from region E1A is shown in Fig. 3. Although none of the clones contains a complete copy of the 12S or 13S mRNA, it was possible to combine our sequence results from the clones with the DNA sequence for the left-hand end of ad5 DNA[6] to deduce the structure of the two mRNAs. Both ad2 and ad5 belong to subgroup C adenoviruses and their transforming regions are nearly identical[9]. From our clones we have collected sequence information comprising more than 500 nucleotides (Fig. 1*d*) and have found only six base-pair changes between ad2 and ad5 DNA (Fig. 3). The sequence at positions where splicing takes place are in all cases identical between ad2 and ad5. Thus, it is possible to combine our sequence information from the clones with the genomic DNA sequence of ad5 (ref. 6) to deduce the structure of the 12S and 13S mRNAs from region E1A. The structure is based on the assumption that the 12S and 13S RNAs have common 5′- and 3′-ends.

The splicing events which must occur during maturation of the 12S and 13S mRNAs result in the deletion of a markedly AT-rich region, between nucleotides 1,113 and 1,228, which contains many stop codons. In this respect the splice has the same function as the splice in the mRNA for the large T-antigen

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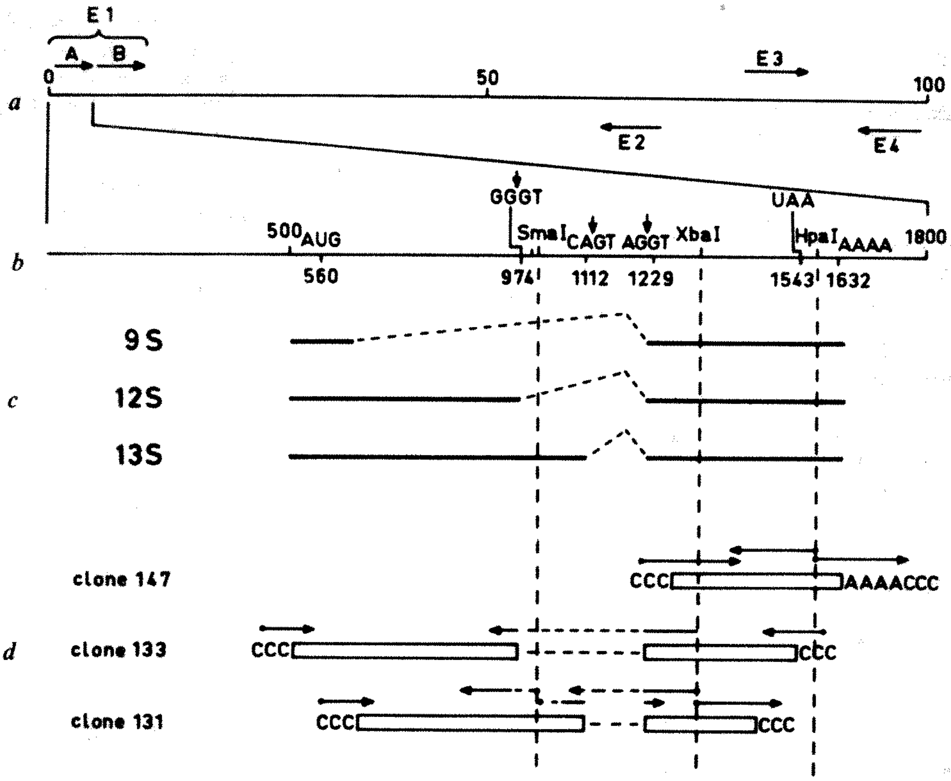

Fig. 1 A schematic drawing of the ad2 genome, showing the four regions which are transcribed early after infection.[18,19] Region E1 contains two promoters which divide it into E1A and E1B (ref. 5). An enlargement of region E1A, indicating cleavage sites for selected restriction enzymes. The locations of the initiator AUG, the termination codon, the poly(A) addition site and the splice points for the 12S and 13S mRNAs are indicated. The nucleotide sequences around the splice points are also shown. Due to the presence of tandem repeats at the splice points (GT in the 13S mRNA and GGT in the 12S mRNA) it is not possible to pinpoint precisely the positions where splicing has taken place. However, by following the 'GT–AG' rule (ref. 20) tentative locations for the splice points can be established and are indicated by arrows. Following this assumption, nucleotide 974 is connected to nucleotide 1,229 in the 12S mRNA, and in the 13S mRNA nucleotide 1,112 is connected to nucleotide 1,229. *c*, structure of the 9S, 12S and 13S mRNAs as determined by electron microscopy[4] and S_1 nuclease mapping[2]. *d*, The boxes indicate sequences which are present in clones 147, 133 and 131. The clones were prepared in the following way. Complementary DNA (cDNA) from 10 µg of poly(A)-containing RNA, prepared as described previously[21] was synthesised with avian myeloblastosis virus (AMV) DNA polymerase, using oligo(dT)$_{12-18}$ as a primer in 0.2 ml of a reaction mixture containing 50 nM Tris-HCl, *p*H 8.2, 10 mM KCl, 75 mM dithiothreitol, 10 mM $MgCl_2$, 4 mM sodium pyrophosphate, 0.05 mM $[\alpha\text{-}^{32}P]$ATP and 0.02 mM dGTP, dCTP and dTTP. The RNA template was removed by hydrolysis in 0.3 M NaOH for 2 h at 50 °C. A second DNA strand was synthesised either with DNA polymerase I or AMV DNA polymerase in the same reaction mixture as described above, without pyrophosphate. The resulting hairpin loop was eliminated by S_1 treatment. The double-stranded cDNA was inserted into the *Pst*I site of the pBR322 plasmid after dG/dC tailing with terminal transferase. The recombinant plasmids were propagated in the DP50 strain of *Escherichia coli* in EK 2 host–vector conditions. The experiments were carried out in the P3 facilities available at the Pasteur Institute, Paris. Details of our cloning procedure will be described elsewhere[15]. Colonies containing ad2-specific sequences were identified by hybridisation[7]. The arrows indicate regions which were sequenced by the Maxam and Gilbert[8] procedure.

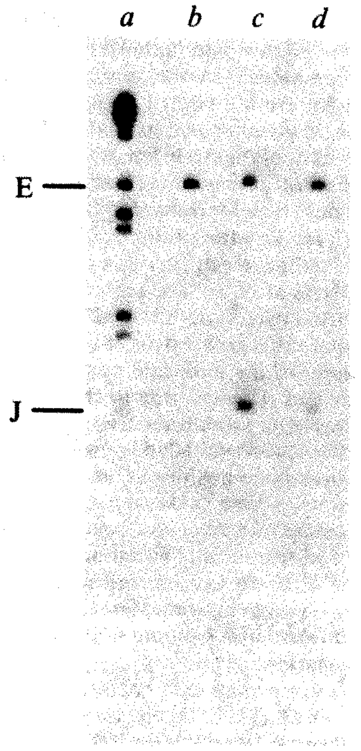

Fig. 2 Hybridisation between ^{32}P-labelled DNA from clones 147 (*b*), 133 (*c*), 131 (*d*) and *Sma*I fragments of ad2 DNA. DNA from the recombinant plasmids was labelled *in vitro* by nick translation. Ad2 DNA was cleaved with *Sma*I and transferred to nitrocellulose[22]. Hybridisation was visualised by autoradiography. ^{32}P-labelled and ad2 DNA was hybridised to indicate the position of all fragments (*a*).

of SV40 (refs 10–12). Also, like the situation for SV40 T-antigen mRNA[13], the 5'- and 3'-parts of both mRNAs will be translated from different reading frames. In this way the coding capacity of region E1A is used in the most efficient way. The polypeptides which are specified by the 13S and 12S mRNAs will be 288 and 242 amino acids long (Fig. 3), with molecular weights of 32,000 and 26,000, respectively, assuming that the AUG at position 560 is used for initiation. Both polypeptides will have identical amino- and carboxy-terminal ends, their only difference being a deletion of 46 internal amino acids from the polypeptide, specified by the shorter mRNA (Fig. 3). Both peptides are unusually rich in proline and glutamic acid (Fig. 3); this may explain why their molecular weights have been overestimated by SDS-polyacrylamide gel electrophoresis[14]. By *in vitro* translation of mRNAs from the extreme left-hand end of the ad2 genome, four polypeptides in the molecular weight range of 42–53,000 have been detected[14]. Thus, more polypeptides have been assigned to region E1A than can be accounted for by the mRNAs which have been observed by electron microscopy[4] and the S_1 nuclease assay[2]. This could be due to post-translational modification or processing of the primary translation products. However, we cannot exclude the possibility that additional mRNAs are specified by region E1A which have such small differences in the positions of their splices that they have escaped detection. Sequence analysis of additional clones is clearly required to resolve this problem. The 3'-end of region 1A mRNAs has been positioned to nucleotide 1,632. However, as two As follow the T in the DNA sequence, it is not possible to predict precisely where the poly(A) addition occurred. We have made a similar observation for the poly(A) addition site in the mRNA for adenovirus polypeptide IX[15] and the presence of two or more As at the poly(A) addition site may be a common feature. Our results do not exclude the presence of

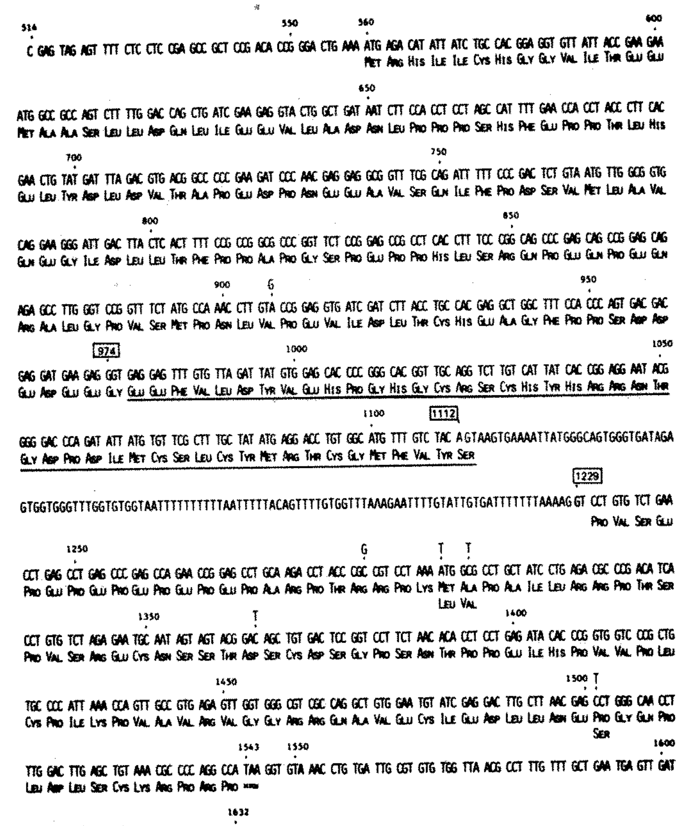

Fig. 3 The proposed structure for the 13S mRNA as deduced from our analysis of the mRNA clones together with the sequence for the left hand end of ad5 DNA[6] (J. Maat *et al.*, personal communication). The sequence corresponds to the 13S mRNA from ad5. Differences between the ad2 and ad5 sequences as noted in our present study are indicated. The 5'-end of region E1A mRNAs is not defined by our cloned sequences. Nucleotide 514 is the first nucleotide present in clone 133. The true 5'-end is probably located a few nucleotides upstream from this position. The sequence which is absent in the 12S mRNA is underlined. The hexanucleotide, present close to the poly(A) junction of eukaryotic mRNAs[16], is indicated by the box. Nucleotides which become connected after splicing are shown in boxes (see also Fig. 1*b*). The nucleotide sequence is indexed according to the method of van Ormondt *et al.*[6].

other poly(A) addition sites for region 1A mRNAs, as only one poly(A) junction has been sequenced so far. This seems, however, unlikely, because a single hexanucleotide sequence, AAUAAA, is present in the DNA sequence[6] and this sequence has been found to precede the poly(A) junctions of all eukaryotic mRNAs[16]. Sequence analysis of clone 133 places the 5'-end of region 1A mRNAs at nucleotide 514 in the sequence of van Ormondt *et al.*[6]. It is, however, conceivable that a few nucleotides are missing from this clone, as the second strand of the cDNA was synthesised by looping back the first strand. Furthermore, a promoter-like sequence, TATTTATA, starts 39 nucleotides upstream from position 514. Such sequences are usually found 23–26 nucleotides preceding the cap-site (D. Hogness, personal communication and ref. 17).

Independent of this investigation, Baker and Ziff (manuscript in preparation) have identified and analysed the capped T_1 oligonucleotide from region E1A mRNAs. Their results indicate that nucleotide 499 in the sequence of van Ormondt *et al.*[6] corresponds to the 5'-end of mRNAs from region E1A. Thus, the first 15 nucleotides of the 12S mRNA are missing from clone 133.

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Inter- and intraspecific variation in restriction maps of *Drosophila* mitochondrial DNAs

D. M. Shah* & C. H. Langley

Laboratory of Animal Genetics, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709

Mitochondrial (mt) DNAs have been observed to be polymorphic for cleavage sites of various restriction endonucleases[1–4]. Although these studies involved a variety of organisms, they were insufficient for the estimation of the level of genetic variation in natural populations. mtDNAs of various *Drosophila* species are heterogeneous in length, with molecular weights (MWs) ranging from 9.90 to 12.35×10^6. The mtDNA molecule of *Drosophila melanogaster* contains a long A+T-rich region representing 25% of the circle length[5,6]. A comparison of mtDNAs from different species of *Drosophila* by electron microscope partial denaturation mapping has shown that the size of the A+T-rich region varies among different species[5]. We have previously presented electron microscope heteroduplex analysis of the mtDNAs of *D. melanogaster*, *Drosophila simulans* and *Drosophila virilis* and showed that the sequence divergence was apparently greatest in the A+T-rich region, whereas the remainder of the molecule was much more evolutionarily conserved. The cleavage map of the *D. melanogaster* mtDNA molecule has been established using various restriction endonucleases[6–8]. The ribosomal RNA genes (rRNAs) and the A+T-rich region have been positioned on this map. We now present the restriction endonuclease maps of the mtDNAs of three species of *Drosophila*. We also report the results of a systematic survey of restriction site variation in the mtDNAs of natural populations of these species and make a preliminary comparison with the level of variation of nuclear genes. Our data suggest that the mitochondrial genome may evolve in a similar way to allozymic variation.

In this study, we have mapped the restriction sites in the mtDNA molecules of *D. virilis*, *D. melanogaster* and *D. simulans* using the enzymes *Eco*RI, *Hin*dIII and *Hpa*II. These enzymes make a small number of cuts in these mtDNAs and are therefore valuable in mapping the cleavage sites and in definitive identification of the conserved sites. mtDNAs were purified by

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*Present address: Department of Microbiology, Cummings Life Science Center, 920 E. 58th Street, University of Chicago, Chicago, Illinois 60637.

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Table 1 Sizes of the restriction fragments of *Drosophila* mtDNAs

	*Hin*dIII			*Hae*III			*Eco*RI			*Hpa*II		
Fragment	*D.m.*	*D.s.*	*D.v.*	*D.m.*	*D.s.*	*D.v.*	*D.m.*	*D.s.*	*D.v.*	*D.m.*	*D.s.*	*D.v.*
A	7.39	7.39	6.14	8.88	6.14	14.85	11.33	7. 80	9.47	9.70	14.24	10.15
B	4.90	4.90	5.61	5.52	5.42		5.24	4.35	5.18	3.46	2.06	5.30
C	4.67	4.67	2.09	3.35	5.42		1.58	1.67	0.83	3.00	1.76	
D	0.43*	0.43*	1.21				0.98	1.36		2.01		
E								1.14				
F								0.91				
G								0.65				
Total	17.39	17.39	15.05	17.93	16.98	14.85	19.13	17.88	15.48	18.17	18.06	15.45

Fragment sizes are given in kilobases. The sizes of the fragments were determined from the agarose gels except as mentioned above. The sizes of *D. melanogaster* and *D. simulans* mtDNAs as determined by electron microscopy are 18.61 and 18.53 kilobases, respectively; that of *D. virilis* mtDNA is 15.86 kilobases. *D.m.*, *D.s.* and *D.v.* refer to *D. melanogaster, D. simulans* and *D. virilis* mtDNAs, respectively.

* Measured from polyacrylamide gels.

two cycles of CsCl–ethidium bromide centrifugation and digested with appropriate restriction endonucleases as described previously[7]. The sizes of the resulting fragments were determined from the calibrated agarose or polyacrylamide gels and by electron microscopy using ΦX174 replication form (RF) II DNA as an internal standard. Table 1 lists the sizes of the *Eco*RI, *Hin*dIII, *Hae*III and *Hpa*II restriction fragments of *Drosophila* mtDNAs. We have determined the relative positions of these restriction fragments on the mtDNA molecules by analysing the products of various double-enzyme digests. Thus, a total of 10 restriction sites were mapped on the *D. virilis* mtDNA molecule, whereas a total of 15 and 17 restriction sites were mapped on the mtDNA molecules of *D. melanogaster* and *D. simulans*, respectively. Figure 1 shows the cleavage maps of these mtDNAs. The cleavage maps of the *D. melanogaster* mtDNA molecule showing the positions of *Eco*RI, *Hin*dIII and *Hae*III sites have been reported previously[6,8]. Our map of *D. melanogaster* mtDNA molecule agrees with the published maps. The distribution of restriction sites is similar in two closely related species, *D. melanogaster* and *D. simulans*, but is markedly different in *D. virilis*, which is more distantly related to the other two species. *D. melanogaster* and *D. simulans* mtDNA molecules are similar in size[7] and share 11 restriction sites. The *D. virilis* mtDNA molecule is 2.75 kilobases shorter and shares only six restriction sites (see Fig. 1). One striking feature of *Drosophila* mtDNA maps is that four restriction sites proximal to the A+T-rich region are conserved in mtDNAs of all three species. Klukas and Dawid[6] have mapped mt-rRNA genes in this region of *D. melanogaster* mtDNA. It is therefore evident that the restriction sites within the rRNA genes of *Drosophila* mtDNAs are conserved. We have also mapped the A+T-rich region of each mtDNA molecule on the cleavage map by partial denaturation mapping and heteroduplex analysis of the *Hin*dIII and *Hae*III fragments of mtDNAs (see Fig. 1). It is evident that the relative positions of the A+T-rich regions are similar in mtDNA molecules of all three species. From the maps presented, it is clear that the A+T-rich region does not contain any restriction sites. *Alu*I, which recognises the sequence 5′ AGCT 3′, cuts *D. melanogaster* and *D. simulans* mtDNA molecules into approximately 18 fragments, but fails to cut the A+T-rich region. This region therefore contains little or no G and C. Using the maximum likelihood estimator of Kaplan *et al.*[9], we have estimated a 6% sequence divergence between *D. melanogaster* and *D. simulans* mtDNAs outside the A+T-rich region and the rRNA genes. However, the restriction map of *D. virilis* mtDNA is completely different from *D. melanogaster* and *D. simulans* mtDNAs outside the rRNA genes so that no further estimations could be made. However, some of the restriction sites surveyed might be appropriately modified in these native mtDNAs, leading to spurious conclusions concerning the presence of the sequence. In the absence of any direct evidence with respect to this question the analysis mentioned above and subsequent analysis of population data (below) cannot take it into account.

Ten isofemale lines of *D. melanogaster* were established simultaneously from a wild collection made in Raleigh, North Carolina, in 1977. Ten isofemale lines of *D. virilis* were also established from two collections made in Sapporo and Moji, Japan, in 1977 (five lines each). Five isofemale lines of *D. simulans* came from a collection made in Mishima, Japan, in 1977. The flies were cultured, the mtDNAs purified from the second and third instar larvae, and restriction sites mapped. Within each isofemale line mtDNA molecules always appeared homogeneous with respect to their restriction maps. Three variable sites were found among *D. melanogaster* lines (see Fig. 1). Figure 2*a* and *b* show the electrophoretic patterns of the restriction enzyme digest of different mtDNAs. These were

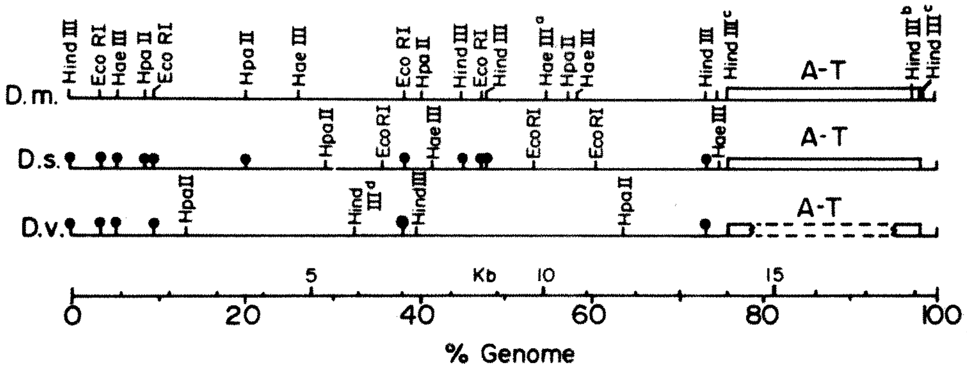

Fig. 1 The *Hin*dIII, *Hae*III, *Eco*RI and *Hpa*II restriction maps of *D. melanogaster* (*D.m.*), *D. simulans* (*D.s.*) and *D. virilis* (*D.v.*) mtDNAs. Units are % of genome and kilobases. Sites with superscripts are variable, as indicated in Fig. 3. The alternative positions of *Hin*dIII^{c} are indicated. *D. melanogaster* and *D. simulans* mtDNAs are 18.6 kilobase pairs in length. *D. virilis* mtDNA has 15.8 kilobase pairs. The reduction in the size of the A+T-rich region in *D. virilis* mtDNA has been indicated by broken lines (= = = =). ● Represents the restriction sites in *D. simulans* and *D. virilis* mtDNAs which are conserved in *D. melanogaster* mtDNA. Circular maps have been linearised to show the conserved restriction sites.

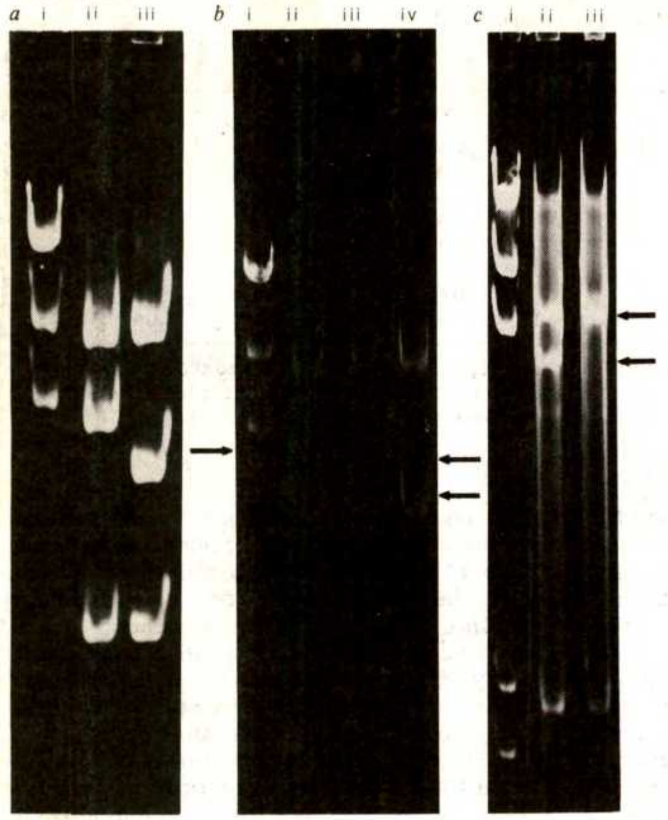

Fig. 2 Restriction analysis of *Drosophila* mtDNAs. *a*, 1.2% agarose gel of *Hin*dIII-digested λ DNA (i), *Hae*III-digested *D. melanogaster* mtDNA without *Hae*III^a site (ii) and with the *Hae*III^a site (iii). *b*, 1.2% agarose gel of *Hin*dIII-digested λ DNA (i), *Hin*dIII-digested *D. melanogaster* mtDNA without sites *Hin*dIII^b or *Hin*dIII^c (ii), with *Hin*dIII^b and without *Hin*dIII^c (iii) and with *Hin*dIII^c (iv). The lack of fluorescence of the middle band is probably due to long poly(dA)·poly(dT) segments. *c*, A 1.2% agarose gel of *Hin*dIII-digested λ DNA (i), *Hin*dIII-digested *D. virilis* mtDNA with the *Hin*dIII^d restriction site (ii) and without the *Hin*dIII^d site (iii). The gels were stained with ethidium bromide and photographed on Polaroid films using short-wave UV source.

distributed among the various lines as shown in Fig. 3*a*. The other variable site occurred among *D. virilis* lines as indicated in Figs 1 and 3*b*. Figure 2*c* shows the two *Hin*dIII patterns observed in *D. virilis*. The two sampling locations gave equal numbers of the two types of *D. virilis* mtDNA. No variation was observed among the lines of *D. simulans*.

Other than these data, the only information available on the intraspecific variability of *Drosophila* mtDNA restriction maps is from the 'standard' lines used in earlier studies. The standard line of *D. melanogaster* (D.m.1) used in our earlier work was collected in Raleigh, but 2 yr earlier than those reported above. The restriction map of this line for the four restriction enzymes is identical to that of the second most frequent class (see Fig. 3*a*). Restriction maps obtained by other laboratories on *D. melanogaster* mtDNA with *Hin*dIII, *Hae*III and *Eco*RI also fall into this class[6,8]. This mtDNA restriction map seems to be widespread and temporarily stable. The *D. virilis* line described in our previous studies[7] was also from Japan but collected 1 yr earlier in Fukuoka. We examined two lines from that collection, one of which had our standard and the other the variant pattern (Fig. 2*c*), suggesting temporal and geographical stability for this polymorphism. Finally, our *D. simulans* standard was derived from Raleigh, whereas our population sample (above) was collected several years later in Japan. The uniformity of the restriction maps in *D. simulans* is in striking contrast to the variability observed in *D. melanogaster*.

We can attempt to answer two questions with these results. The first concerns the significance of the interspecific differences in restriction map variability and the second compares the amount of variation in mtDNA and nuclear DNA. Unfortunately, there has been no systematic survey on the population level of restriction map variation in nuclear DNA. To investigate these matters, we must first determine a metric of restriction map variability that lends itself to analysis (the product of the population size and mutation rate).

Fundamentally, the population genetics of restriction maps is a special case of the 'finite sites model' (ref. 9). Also, the finite sites model is well approximated by the 'infinite alleles model' when the amount of variation relative to that possible is small[10]. Assuming the non-recurrent neutral mutation, finite population model[11] (infinite alleles model), we can estimate the pertinent parameter by two methods. If N_e is the effective population size (number of gene copies), and μ the mutation rate[11], either we can set $1/(2N_e\mu+1)$ equal to the 'expected homozygosity', that is, the sum of squared frequencies[11], or $2N_e\mu$ can be estimated by the maximum likelihood method of Ewens[12]. Estimates from these methods do not differ greatly (*D. melanogaster*, 3.6 or 2.0; *D. virilis*, 1.8 or 0.8; *D. simulans*, 0.0). When these values are tested using the sampling statistics of Ewens[12], the three species do not differ significantly.

To compare the mtDNA and allozyme data, each must be converted into a common unit of genetic variation. In our estimates of $2N_e\mu$, the mutation rate, μ, is the rate per generation of nucleotide changes that create new restriction sites or destroy pre-existing ones, that is, the rate of nucleotide substitutions that create new restriction maps. As pointed out above, if the extant variation is small (as it is in these cases) virtually all site changes will be new. If we assume a random nucleotide sequence and random mutation, the proportion of nucleotide substitutions that change restriction sites can be estimated[7]. In our case, it is 0.02 for the region of 12,000 base pairs excluding the A+T-rich DNA and that coding for the mt-rRNA. We exclude these regions because the A+T-rich region contains no restriction sites (GC content too low) and the rRNAs are much more conservative evolutionarily. On a per nucleotide basis, $2N_e\mu$ is then between 0.008 $[2.0/(0.02\times12{,}000)]$ and 0.0, with a mean of 0.003.

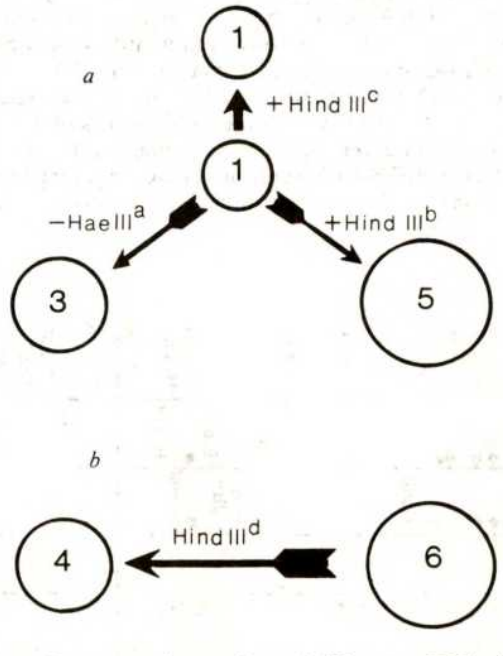

Fig. 3 *a* Represents the numbers of different mtDNAs found among the *D. melanogaster* isofemale lines and the substitutional paths between them. *b* Is a similar presentation of the mtDNAs of the *D. virilis* isofemale lines.

As there are no comparable estimates of restriction map variability in nuclear DNA, we must attempt to use electrophoretic data on population variability. Starting with an estimate of 0.04–0.14 for expected heterozygosity[13,14], we estimate $2N_e\mu$ to be 0.04–0.16 (ref. 11). If we assume that one-quarter of all nucleotide substitutions change the amino acid[15] and one-third of amino acid substitutions change the electrophoretic mobility, then μ in the above estimate is 0.08 of the total nucleotide mutation rate. Assuming 1,000 nucleotide positions per protein, this yields an estimate for $2N_e\mu$ of 0.0005–0.002 on a per nucleotide basis. Note that the electrophoretic techniques of detection of genic variation in proteins are more sensitive than the use of these four restriction enzymes (8% compared with 2% of the nucleotide substitutions). This is partly due to the low GC content of *Drosophila* mtDNAs.

Thus, we estimate $2N_e\mu$ for mtDNA at 0.003 (0–0.008) and for allozyme loci at 0.0005–0.002. Even considering the fact that N_e should be twice as large for nuclear (diploid) genes, there do not seem to be any significant differences in genetic variation between allozymes and mtDNAs. Whether this picture will survive more detailed scrutiny is unknown, but it seems likely that the quality of data on molecular genetic variation in natural populations will change significantly.

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Structure of the region of the replication origin of the *Bacillus subtilis* chromosome

Motoharu Seiki, Naotake Ogasawara & Hiroshi Yoshikawa

Cancer Research Institute, Kanazawa University 13-1, Takaramachi, Kanazawa, 920, Japan

The chromosome of a Gram-positive bacterium, *Bacillus subtilis*, binds tightly to the cell membrane[1–3] probably through a DNA–protein–RNA complex formed specifically near the origin of replication[4,5]. Characterisation of this complex may reveal the role of the cell surface in regulating the initiation of DNA replication in bacteria. As a first step towards this goal, we have constructed a map of the restriction enzyme cleavage sites at the region of the origin of replication of the *B. subtilis* chromosome. Adopting the methodology originally used by Marsh and Worcel in *Escherichia coli*[6], the region of the origin of replication of the chromosome was labelled specifically with a radioisotope which was then used to characterise DNA fragments produced by various combinations of restriction enzyme. In the study reported here, cleavage sites by three enzymes were identified within the chromosomal fragment of molecular weight 4×10^7 at the origin region.

A thymine-requiring mutant of *B. subtilis*, CRK2000 (*trp, leu, thy*)[7], and its *ts-dna* mutant derivative, CRK2001 (*trp, leu, thy, din*^{ts})[8] which is defective in the initiation of DNA replication at 47 °C, were used to label the DNA at the origin of replication. Initiation of the replication cycle was synchronised either by germinating spores in the absence of thymine for at least 2 h or by incubating the ts-dna mutant cells at 47 °C for 1 h followed by incubation at 30 °C in the absence of thymine. In both cases addition of ^{3}H-thymidine (dThd) of high specific activity was assured to initiate chromosomal replication from the origin of replication and to label the origin region of the chromosome selectively[4]. The labelled DNA was purified and digested with

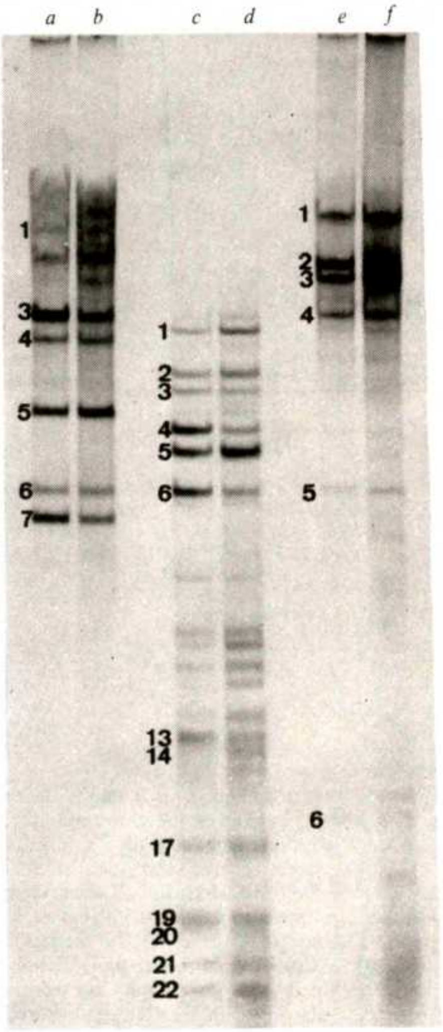

Fig. 1 Restriction endonuclease analysis of DNA, pulse labelled during the initiation of replication. Spores of *B. subtilis* CRK2000 (*leu, trp, thy*)[7] were germinated at 37 °C in a synthetic medium, GM11 (ref. 1), in the absence of thymine for 150 min, and then labelled with ^{3}H-dThd (Radiochemical Centre, Amersham), 10 μCi per 50 ng per ml, for 1 min (*b, d, f*). Novobiocin (Boehringer, Mannheim, 100 μg ml^{-1}) was added to a separate sample 5 min before the spores were labelled with ^{3}H-dThd for 8 min (*a, c, e*). Novobiocin reduced the rate of DNA replication to 7.5% of the control rate. The labelling was stopped by pouring the culture into an equal volume of ethanol–phenol (75% ethanol, 2% phenol in 21 mM Na-acetate buffer, *p*H 5.1, and 2 mM EDTA). Cells were collected by centrifugation, lysed with 1 mg ml^{-1} lysozyme in a lysis buffer (20 mM Tris-HCl, *p*H 8.1, 100 mM KCl, 1 mM EDTA) for 30 min and then treated with 1% Sarkosyl (Geigy) and 500 μg ml^{-1} pronase E (Kaken Kagaku). DNA was extracted and purified by repeating the phenol extraction and precipitation by ethanol in the lysis buffer. Purified DNA was digested wtih *Bam*HI (prepared as described previously[11]) (*a, b*), *Eco*RI (Miles) (*c, d*) or with *Sma*I (*e, f*) according to standard methods. Fragments were separated by electrophoresis in 0.7% agarose gel (Seakem) at 125 V for 16 h and detected by fluorography as reported by Marsh and Worcel[6].

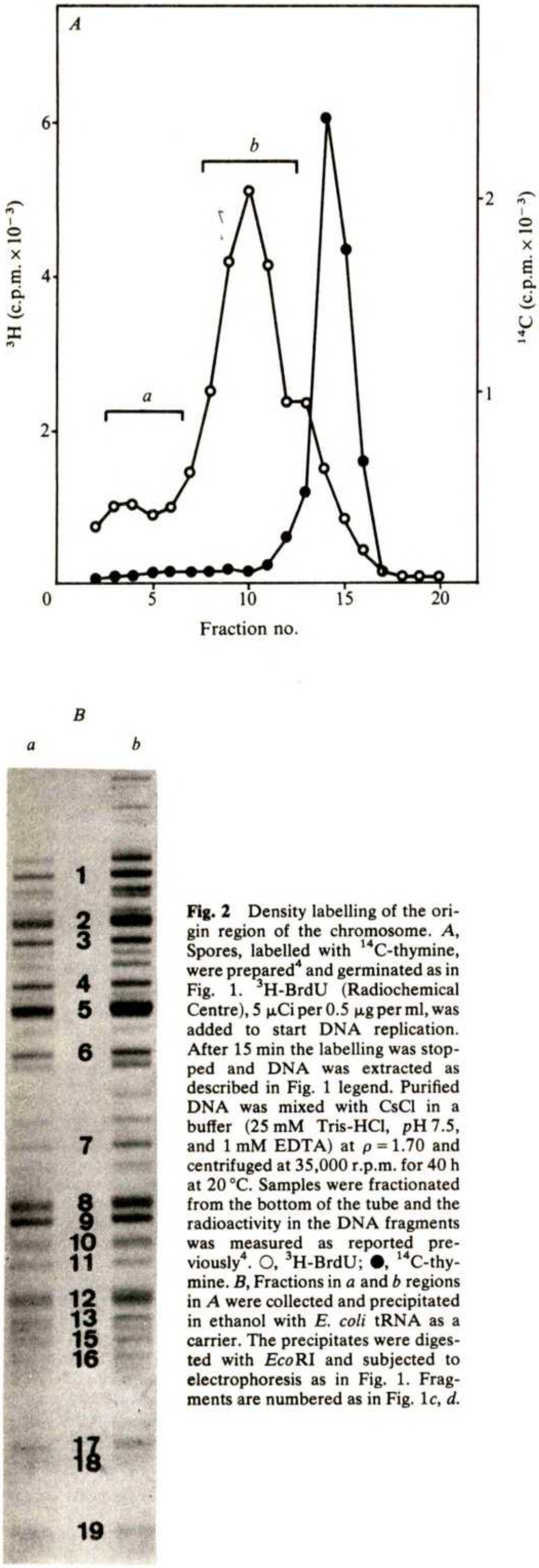

Fig. 2 Density labelling of the origin region of the chromosome. *A*, Spores, labelled with ^{14}C-thymine, were prepared[4] and germinated as in Fig. 1. ^{3}H-BrdU (Radiochemical Centre), 5 μCi per 0.5 μg per ml, was added to start DNA replication. After 15 min the labelling was stopped and DNA was extracted as described in Fig. 1 legend. Purified DNA was mixed with CsCl in a buffer (25 mM Tris-HCl, *p*H 7.5, and 1 mM EDTA) at $\rho = 1.70$ and centrifuged at 35,000 r.p.m. for 40 h at 20 °C. Samples were fractionated from the bottom of the tube and the radioactivity in the DNA fragments was measured as reported previously[4]. ○, ^{3}H-BrdU; ●, ^{14}C-thymine. *B*, Fractions in *a* and *b* regions in *A* were collected and precipitated in ethanol with *E. coli* tRNA as a carrier. The precipitates were digested with *Eco*RI and subjected to electrophoresis as in Fig. 1. Fragments are numbered as in Fig. 1*c*, *d*.

various restriction endonucleases, and the resultant ^{3}H-labelled fragments were analysed by agarose gel electrophoresis and fluorography.

Several discrete bands of fragments were detected in all enzyme digests. Figure 1 shows fragments produced by *Sma*I, *Eco*RI and *Bam*HI of the pulse-labelled DNA during synchronous initiation by germinating spores. The number of detectable bands increased with the length of the labelling period (details of the time course are reported in the accompanying paper[9]). Essentially identical fragment patterns were obtained when DNA was labelled during the synchronous initiation by *ts-dna* mutant (data not shown). To confirm that the label was incorporated by semi-conservative replication and not by a repair-type synthesis, ^{3}H-bromodeoxyuridine (BrdU) was added in place of ^{3}H-dThd to the germinating spores. A completely replicated DNA was collected by CsCl equilibrium density centrifugation and its restriction enzyme fragments were analysed. Figure 2 shows that (1) a short pulse with ^{3}H-BrdU resulted in a density shift, indicating semi-conservative replication, and (2) ^{3}H-BrdU-labelled fragments of the *Eco*RI digest were identical to those of ^{3}H-dThd-labelled DNA.

Major bands of Fig. 1 were used for further analysis to construct the cleavage map. Each band produced by one enzyme was extracted, purified and then digested with the other two enzymes to make a thorough comparison between the three groups of fragments. Results of the analysis are summarised in Table 1 and a map of cleavage sites was constructed and is shown in Fig. 3. First, the relative location of fragments S6 and S2 were determined because fragment B3 overlapped these two *Sma*I fragments. As most of fragment B5 was derived from fragment S2, fragments B3 and B5 must be contiguous to each other. The third and largest *Sma*I fragment, S1, contained one *Bam*HI site, thus producing two *Bam*HI fragments, B1 and the major portion of B7. When fragment B7 was digested by *Sma*I three fragments were produced. The largest of these, of MW 2.85×10^{6}, corresponded to one of the two *Bam*HI fragments derived from S1, whereas the smallest fragment, of MW 0.2×10^{6}, corresponded to the smaller one of the two fragments derived from fragment S6 by *Bam*HI digestion. These results indicated that fragments S1 and S6 were contiguous to each other, with a fragment of 0.5×10^{6} MW inserted between them. Therefore, the relative locations of the *Sma*I fragments are S1–0.5×10^{6} MW fragment–S6–S2. From this result the order of the *Bam*HI fragments was deduced as B1–B7–B3–B5.

Once the cleavage sites of *Sma*I and *Bam*HI were mapped, *Eco*RI sites could be assigned as follows. Fragments E4 and E22 could be assigned as they were derived from one of the four *Bam*HI fragments and contained *Sma*I sites. In the same way fragments E6, E17 and E27 were located because they were derived from one of the three *Sma*I fragments and contained one *Bam*HI site. The space between fragments E6 and E22 matched precisely with E19, which is derived from fragment B7. Fragment E13 was located between E4 and E17 in the same manner. Fragments E5 and E20 can be mapped at any place within fragment B1. However, we set these fragments as contiguous to E6 because they were replicated much earlier than fragment E1. Relative positions of fragments E14, E21 and E26, all derived from B5, have not been determined. One of the major early replicating *Sma*I fragments, S3, whose *Bam*HI and *Eco*RI sites have been mapped as in Fig. 3, is most probably located close to S2. Assuming B5 and B6 to be contiguous to each other, there will be an additional *Sam*I fragment of MW $\sim 1.8 \times 10^{6}$, containing one *Bam*HI site, situated between fragments S2 and S3. A fragment of the corresponding MW can be seen above fragment S6 in Fig. 1. Therefore, it is most likely that fragments B5 and B6 are contiguous and so the map in Fig. 3 can be drawn continuously. However we cannot be certain of this until the additional, small *Sma*I fragment is fully characterised. The origin of replication is located within fragment B7, as described in the accompanying paper.

We have reported recently that a DNA segment near the origin of replication formed a specific complex (S-complex) with

Table 1 Results of enzyme analysis

First cleavage	Fragment no.	MW (×10^6)	Second cleavage *Bam*HI	Second cleavage *Sma*I	Second cleavage *Eco*RI
*Bam*HI	1	15		ND	ND
	3	8.2		6.7(S2), 1.3(S6)	4.7(E4), 1.9(E13), 0.86(E17), 0.40(E27)
	4	7.2		Id	4.9(E2), 2.1(E3)
	5	5.1		4.5(S2), 0.5	1.80(E14), 0.95(E21), 0.85, 0.55(E26), 0.45(E17) 0.30
	6	3.8		2.5(S3), 1.3	3.3(E3), 0.4
	7	3.4		2.85(S1), 0.50, 0.2(S6)	1.35(E6), 1.05(E19), 0.85(E22), 0.15(E27)
*Sma*I	1	18	15(B1), 2.9(B7)		7.9(E1), 4.3(E5), 3.7(E6), 1.10(E19), 1.05(E20) 0.30(E22)*
	2	11.6	7.0(B3), 4.6(B5)		3.8(E4), 1.9(E13), 1.8(E14), 1.4(E17), 0.95(E21) 0.56(E26), 0.37, 0.3*
	3	10.5	7.2(B4), 2.7(B6), 0.4		5.8(E2), 4.6(E3)
	6	1.5	1.3(B3), 0.2(B7)		0.98(E4), 0.54(E27), 0.06(E22)*
*Eco*RI	1	8.0	ND	ND	
	2	6.20	5.0(B4), 0.7	ND	
	3	5.70	3.4(B6), 2.0(B4)	ND	
	4	4.70	Id(B3)	3.70(S2), 1.0(S6)	
	5	4.30	Id(B1)	Id(S1)	
	6	3.70	2.2(B1), 1.3(B7)	Id(S1)	
	13	1.90	Id(B3)	Id(S2)	
	14	1.80	ND	ND	
	17	1.35	0.90(B3), 0.45(B5)	Id(S2)	
	19	1.10	Id(B7)	Id(S1)	
	20	1.05	Id(B1)	Id(S1)	
	21	0.95	ND	ND	
	22	0.86	Id(B7)	0.50, 0.30(S1)*, 0.06(S6)*	
	26	0.56	ND	ND	
	27	0.54	0.40(B3), 0.14(B7)	Id(S6)	

Fragment numbers are the same as those in Fig. 1. Each fragment was cut out from the gel and DNA was extracted with 4 M KI and purified by passing through a hydroxyapatite column as reported previously[10]. Each DNA sample was then digested with the other two enzymes and analysed by the same electrophoresis method as described in Fig. 1 legend. The MW of each fragment was determined by making parallel electrophoretic runs for ^{3}H-labelled standard fragments of known MW (*Eco*RI and *Hin*dIII digests of λ DNA). Id (indigestible) indicates that the fragment did not contain sites cleaved by the second enzyme concerned. ND, Not determined. By comparing each pair of doubly digested fragments of the same enzyme combinations but in reverse order, we determined the second source of fragment to which a given fragment belongs; these are indicated in parentheses.

*Fragments assumed to occur in the electrophoresis but not detected in the conditions used.

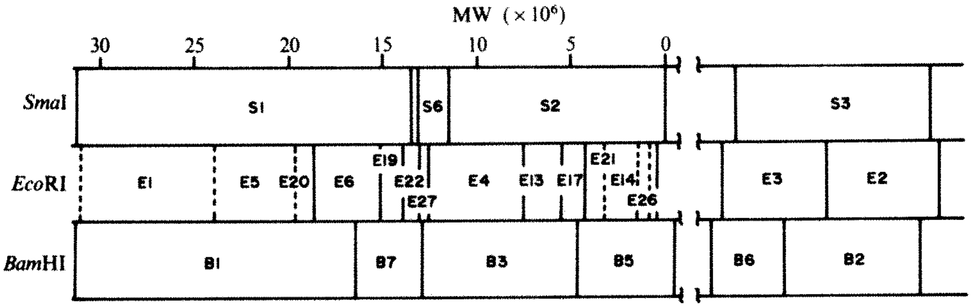

Fig. 3 A map of restriction enzyme cleavage sites near the origin of replication. Relative location of the fragments in Fig. 1 and Table 1 were determined as discussed in the text. To indicate the MW of the fragments, the right hand edge of the *Sma*I-2 fragment was taken as a reference point.

proteins and RNA[3,4]. Asymmetric location of the DNA fragments in the S-complex at one side of the replication origin was assumed by comparing restriction enzyme fragments of this DNA with those of the pulse-labelled DNA at the region of the origin of replication[4,5]. The present mapping data are consistent with the asymmetric location of the S-complex. Judging from the size of the fragments, there are homologues of the E1 and E5 fragments in the DNA from the S-complex, while no fragments to the right hand side of E20 have homologues in the complex DNA. This result is also consistent with the genetic evidence for the asymmetric association of the origin region of the *B. subtilis* chromosome with the cell membrane[3,4].

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Effect of novobiocin on initiation of DNA replication in *Bacillus subtilis*

Naotake Ogasawara, Motoharu Seiki & Hiroshi Yoshikawa

Cancer Research Institute, Kanazawa University 13-1, Takaramachi, Kanazawa 920, Japan

The initiation of DNA replication of small replicons *in vitro* involves conformational changes in the whole DNA molecule or in the region near to the replication origin[1–4]. One striking finding has been the role of DNA gyrase (that is, the necessity for supercoiled structure) in the initial stage of ColE1 replication *in vitro*[5,6]. However, little is known about the effect of gyrase on the initiation of replication of bacterial chromosomes *in vivo*. We have constructed a map of cleavage sites of restriction enzymes at the region of the origin of replication of the *Bacillus subtilis* chromosome (accompanying paper[7]). This has now enabled us to examine the effect of novobiocin, a selective inhibitor of DNA gyrase[8], on the replication of the specific chromosomal segments near the origin and to seek a possible role for the gyrase in the initiation of chromosomal replication. We have found that only a limited segment of the chromosome at the origin region was replicated in the presence of novobiocin. This effect allowed us to locate the site of the origin of replication to within a DNA fragment of molecular weight 3.4×10^6.

We used a spore germination of a thymine-requiring mutant of *B. subtilis*, CRK2000 (*leu*, *trp*, *thy*)[9], to synchronise the initiation of replication. The mutant spores were germinated in a synthetic medium, GM11 (ref. 10), in the absence of thymine for 150 min; ^{3}H-thymidine (dThd) was then added to initiate replication synchronously.

Figure 1 shows that replication started immediately after the addition of dThd. Therefore, the region which was labelled early probably contained the region of the origin of replication. When novobiocin was added at various concentrations 60 min before dThd, DNA replication was markedly inhibited, in a manner dependent on the inhibitors concentration. At each concentration replication proceeded linearly at reduced rates. When novobiocin was added at various times during the thymine-free germination and thymidine added later at the 150th min to all samples, varied effects of the drug were observed. Figure 1*b* shows that the initiation of replication was further reduced when novobiocin was added during the first 45–50 min after the onset of germination. After 45–50 min the reduced rates became fairly constant, depending only on the novobiocin concentration. As DNA replication was initiated synchronously at the 45–50th min during germination in the presence of thymidine, the initiation potential (the cell's ability to initiate replication) was assumed to be formed during the first 45 min. Data in Fig. 1*b* suggest an inhibitory effect of novobiocin on the formation of the initiation potential during germination.

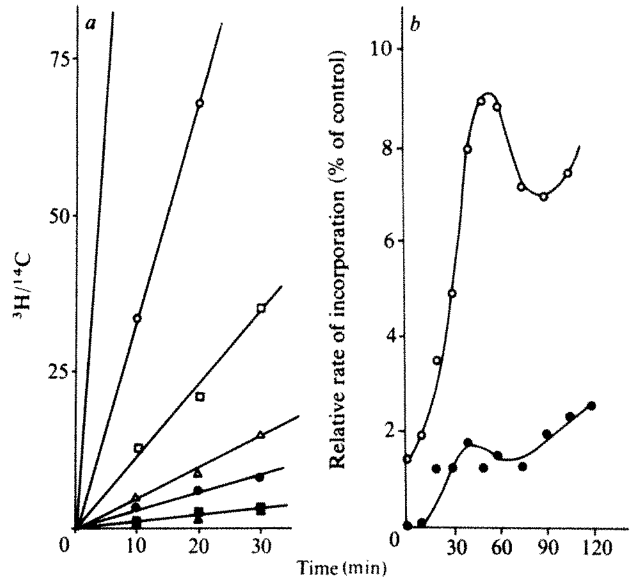

Fig. 1 The initiation of DNA replication in the presence of novobiocin. *a*, spores of *B. subtilis* CRK2000 (*leu*, *trp*, *thy*)[9] were labelled with ^{14}C-thymine as reported previously[9]. The labelled spores were germinated in a synthetic medium GM11 (ref. 10), in the absence of thymine after heat activation at 80 °C for 15 min. After 90 min, novobiocin (Boehringer Mannheim) was added at various concentrations and at the 150th min ^{3}H-dThd (Radiochemical Centre, Amersham), 5 μCi per 75 ng per ml, was added to initiate DNA replication. Thereafter, 1.0-ml samples were taken at the times indicated and precipitated in 10% trichloracetic acid after incubation in 0.2 M KOH for 30 min at 80 °C. The precipitates were collected on a glass fibre filter and radioactivity were measured in a Beckman scintillation counter LS7000. Incorporation of ^{3}H-dThd was normalised by ^{14}C-activity in the spore. –, No drug; –○–, 2.5 μg ml^{-1}; –□–, 5 μg ml^{-1}; –△–, 7.5 μg ml^{-1}; –●–, 10 μg ml^{-1}; –■–, 20 μg ml^{-1}; –▲–, 40 μg ml^{-1} novobiocin. *b*, The ^{14}C-labelled spores were germinated as in *a* except that novobiocin was added at various times during the thymine-free germination. ^{3}H-dThd, 5 μCi per 75 ng per ml, was added to all samples at the 150th min. The rate of incorporation was measured for each sample from the slope of linear incorporation as in *a*. It was then normalised by the initial rate of the control sample and plotted against the time of novobiocin addition. –○–, 5 μg ml^{-1}; –●–, 10 μg ml^{-1} novobiocin.

To examine the nature of this reduced replication in the presence of novobiocin, chromosomal segments which were replicated in the presence of novobiocin were identified by digesting ^{3}H-labelled DNA with restriction endonucleases and then analysing the resulting fragments by agarose gel electrophoresis and fluorography. Figure 2 shows that only a limited number of *Eco*RI fragments were labelled when 10 μg ml^{-1} novobiocin was used. The number of labelled fragments increased more slowly with the lower concentration, 5 μg ml^{-1}. The quantitative measurement of the label in each fragment is shown in Fig. 3*A*, which indicates that fragments were labelled in a definite order in the presence of novobiocin. Thus, fragment E19 was the first to be labelled, followed by fragments E22, E27, E6, E20, E5 and so on. Identical results were obtained with *Bam*HI fragments. Figure 2 also shows that fragment B7, which contained fragment E19, was replicated first, followed by fragments B3 and B5.

Comparing these results with the map of the restriction sites near the origin of replication (see accompanying paper), we estimated time courses of initiation and replication in the presence and absence of novobiocin. The results demonstrated a bidirectional replication starting within fragment B7. Furthermore, it shows clearly that only a limited region around the origin was replicated in the presence of 10 μg ml^{-1} novobiocin. The remarkably high level of incorporation into fragments E19 and E22 was not due to a premature re-initiation, because chloramphenicol had no effect on this level when added 60 min before the isotope. This indicates that some cells replicated to lengths of only $1.0–1.5 \times 10^6$ MW in the presence of the drug. When germinating spores were labelled for short times in the absence of novobiocin, the labelling patterns obtained were essentially the same as those with novobiocin. However, the rate of elongation was too fast to determine the labelling sequence among early replicating fragments other than fragments E19, E22 and E27 (Fig. 3). The unusually high level of labelling in fragment E5 might have been due to its contamination by one or two other fragments which were not replicated in the presence of novobiocin. This is further suggested by the finding that a part of fragment E5 from the control sample was digested by *Bam*HI, whereas fragment E5 from the novobiocin sample had no *Bam*HI site. Also, in certain conditions, the control fragment E5 split into two or more bands.

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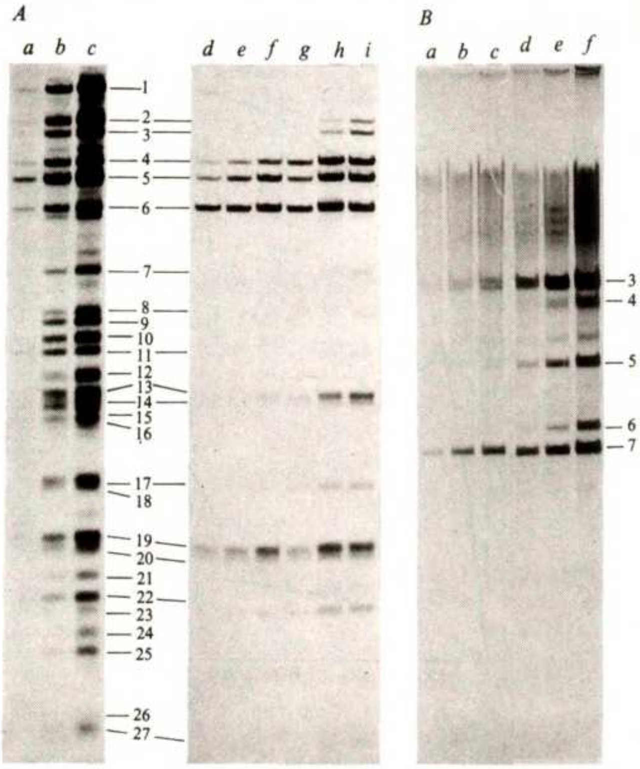

Fig. 2 Analysis by restriction endonucleases of DNA, pulse labelled during the initiation of replication in the presence of novobiocin. Spores were germinated as in Fig. 1. Novobiocin and ^{3}H-dThd, 10 μCi per 50 ng per ml, were added at the 90th and 150th min, respectively. The labelling was stopped by pouring the culture into an equal volume of ethanol–phenol (75% ethanol, 2% phenol in 21 mM Na-acetate buffer, *p*H 5.1, and 2 mM EDTA). Cells were collected by centrifugation, lysed with 1 mg ml^{-1} lysozyme in a lysis buffer (20 mM Tris-HCl, *p*H 8.1, 100 mM KCl, 1 mM EDTA) for 30 min and then treated with 1% Sarkosyl (Geigy) and 500 μg ml^{-1} pronase E (Kaken Kagaku). DNA was purified by repeating the phenol extraction and precipitation by ethanol in the lysis buffer. The purified DNA was digested with *Eco*RI (Miles) or *Bam*HI (prepared as reported previously[11]) using standard methods, and subjected to electrophoresis in 0.7% agarose gel and detected by fluorography as described by Marsh and Worcel[12]. *A*, The patterns for *Eco*RI digests. *a–c* Are DNA samples labelled for 45, 90 and 135 s, respectively, without the drug. *d–f* Are DNA samples labelled for 10, 20 and 30 min with 10 μg ml^{-1} novobiocin. *g–i* Are DNA samples labelled for 10, 20 and 30 min with 5 μg ml^{-1} novobiocin. *B*, The patterns for *Bam*HI digests. *a–c* Are samples labelled for 5, 10 and 15 min with 10 μg ml^{-1} novobiocin. *d–f* Are samples labelled for 5, 10 and 15 min with 5 μg ml^{-1} novobiocin.

The experimental results presented here indicate that novobiocin had two types of effect on DNA replication. First, it inhibited formation of the cell's potential ability to initiate DNA replication. This may be a direct effect on DNA structure or it may act indirectly through inhibition of RNA synthesis. Second, it permitted a limited amount of replication at a specific segment of the chromosome near the origin of replication, once the initiation potential had been formed in the cell.

These results suggest that the conformational changes in the chromosome, due in part to the function of DNA gyrase, is essential both for initiation and for further elongation even after the proper conformation has been formed in the cell. These effects of novobiocin on chromosomal replication resemble those of gyrase on *in vitro* replication of ColE1 plasmid[5,6].

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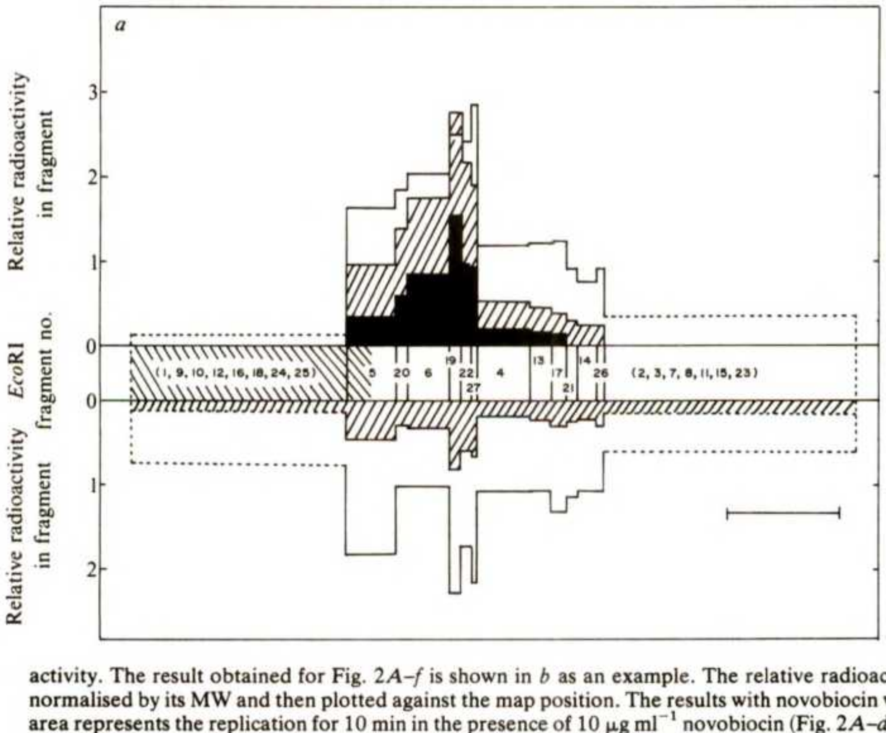

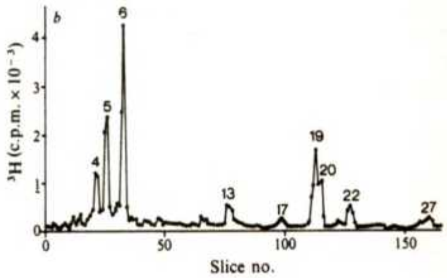

Fig. 3 *a*, A schematic representation of the time course of replication in the presence or absence of novobiocin. The *Eco*RI fragments numbered in Fig. 2*A* were linearly arranged according to the map for restriction enzyme cleavage sites near the origin of replication. The fragments outside E5 and E26 were divided into two groups depending on whether or not a given fragment found its homologue in the DNA in the S-complex[13,14]. Relative locations in each group have not been determined. The hatched area on the map shows the location of the DNA in the S-complex[13,14]. Each agarose gel in Fig. 2*A* was cut in strips 1 mm wide and put in a scintillation mixture to measure ^{3}H activity. The result obtained for Fig. 2*A–f* is shown in *b* as an example. The relative radioactivity under the area of each fragment peak was normalised by its MW and then plotted against the map position. The results with novobiocin were drawn above the fragment map. The shaded area represents the replication for 10 min in the presence of 10 μg ml^{-1} novobiocin (Fig. 2*A–d*). The hatched area represents the replication for 30 min with 10 μg ml^{-1} novobiocin (Fig. 2*A–f*). The open area represents the replication for 30 min with 5 μg ml^{-1} novobiocin (Fig. 2*A–i*). Control samples are shown below the map. The hatched area represents the replication for 45 s (Fig. 2*A–a*) and the open area replication for 90 s (Fig. 2*A*(*b*) both in the absence of novobiocin. The dotted line shows the average radioactivities within the group of fragments shown in parentheses.

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Ribonucleotides in DNA newly synthesised in 3T6 cells *in vivo*

Jacek Kowalski & David T. Denhardt

Department of Biochemistry, McIntyre Medical Sciences Building, McGill University, 3655 Drummond Street, Montreal, Quebec, Canada H3G 1Y6

Within the field of DNA replication, considerable interest has focused in recent years on the mechanism of initiation of synthesis of DNA molecules. *In vitro* replication systems from *Escherichia coli* have been instrumental in uncovering a priming function for ribonucleotides on the earliest intermediates of DNA polymerisation *in vitro* and in identifying the proteins involved[1-3]. *In vitro* replication systems from mammalian cells that permit the use of the phosphate-transfer method for detection of RNA-DNA junctions as well as direct labelling of the RNA moiety of the molecules have suggested a similar role for ribonucleotides in DNA synthesis in eukaryotes[4-8]. However, the existence of this mechanism in mammalian cells *in vivo* has not been established. Here we report the first evidence that a significant proportion of the earliest intermediates in mammalian DNA polymerisation *in vivo* do, in fact, possess ribonucleotides, presumably because their synthesis was initiated with one or more ribonucleotides.

In *E. coli* several types of experiment have been carried out with nascent DNA from intact cells. The direct approach uses purification of nascent DNA molecules, labelling of their 5'-ends with ^{32}P using polynucleotide kinase, and subsequent demonstration of the release of 2'(3'), 5'-[^{32}P]-ribonucleoside diphosphates on alkaline hydrolysis[9]. We used this approach to study DNA synthesis in mammalian cells, and found that, in addition to difficulties due to the presence of contaminating RNA molecules not covalently attached to DNA, the presence of a large number of small non-nascent DNA molecules of uncertain origin obscured an adequate demonstration of ribonucleotides at the 5'-ends of nascent molecules[10,11]. An alternative approach involves alkaline hydrolysis of nascent DNA molecules phosphorylated at the 5'-end with polynucleotide kinase. The appearance of 5'-OH termini, on alkaline hydrolysis of the nascent polynucleotide is assayed by labelling with ^{32}P using polynucleotide kinase[9,12], or by digestion of the molecules with the 5'-OH-specific exonuclease, spleen phosphodiesterase[9,13]. In *E. coli*, there is good evidence that some of the nascent DNA molecules are initiated with ribonucleotides *in vivo*. *Physarum* is the only eukaryote in which evidence (by phosphate transfer) for RNA priming *in vivo* has been obtained[14]. Earlier experiments using differences in buoyant density as a measure of covalent linkage of RNA to nascent DNA[15,16] have been shown to be subject to artefact[17]. We have examined nascent DNA molecules synthesised in intact murine 3T6 cells for the presence of ribonucleotides by the method which uses spleen exonuclease.

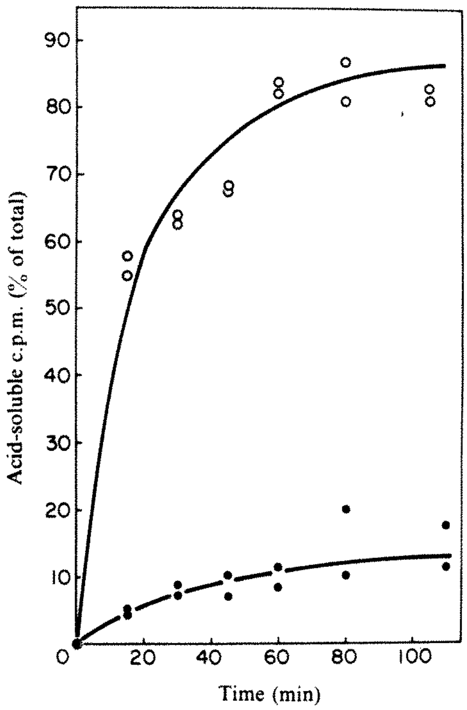

Fig. 1 Time course of digestion of DNA by spleen exonuclease. DNA uniformly labelled with ^3H-dThd and extracted from 3T6 cells with SDS was deproteinised by phenol–chloroform extraction. Heat-denatured DNA was separated from RNA by chromatography on nitrocellulose[10]. The material was then sonicated extensively and fractionated on a neutral sucrose gradient. Fractions containing DNA 1,200–2,400 nucleotides long were pooled. After re-precipitation with isopropanol the DNA (300 nmol nucleotide per ml in 50 mM Tris-Cl, *p*H 8) was heat denatured. One half was incubated with bacterial alkaline phosphatase purified as previously described[9] at a concentration of 1 unit ml^{-1} at 65 °C for 1 h. The other half was exposed to T4 polynucleotide kinase (purified as previously described[9]) and ATP in 50 mM Tris-Cl (*p*H 8.1), 2 mM potassium phosphate, 10 mM $MgCl_2$ and 20 mM 2-mercaptoethanol for 60 min at 25 °C. The amount of ATP required in the reaction mixture was determined by estimating the number of 5'-ends from the concentration of nucleotide and the size of the DNA. ATP was added in a 500-fold molar excess over the number of 5'-ends in the sample. Both kinase and phosphatase-treated portions were then made 20 mM in EDTA, 100 mM in NaCl and 30 μg ml^{-1} in SDS extracted once with phenol–chloroform (1:1) and precipitated with isopropanol. DNA redissolved in water was adjusted to the following conditions: 150 mM CH_3COONa (*p*H 5.5), 10 mM EDTA, 50 mM Na_2SO_4, 30–100 μg ml^{-1} DNA. The DNA was heated at 100 °C for 20 s. Spleen exonuclease, purified as described by Bernardi and Bernardi[18], was then added to a concentration of 33 μg ml^{-1} and incubated with the DNA at 45 °C for the time indicated. Each incubation, in duplicate, was then made 400 μg ml^{-1} in salmon sperm DNA and 10% in trichloroacetic acid. After 20 min at 4 °C, it was centrifuged at 16,000*g* for 10 min. The precipitate was washed once with 10% trichloroacetic acid and redissolved in 0.2 M NaOH at 100 °C. Radioactivity in the supernatant and precipitate was determined in an aqueous scintillation counting cocktail after neutralisation. ○, Phosphatase-treated DNA; ●, kinase-treated DNA.

Spleen exonuclease was purified from porcine spleen by the procedure described by Bernardi and Bernardi[18]. The specificity of the enzyme for 5'-OH-terminated DNA is shown in Fig. 1. The maximal extent of digestion of 5'-OH DNA was 85-90%. The enzyme shows a marked preference for single-stranded molecules[18] and the residual resistance is probably due to the presence of secondary structure in the DNA. In these conditions 5'-phosphoryl DNA was degraded to the extent of about 15%. We suspect that this is the result of the presence of small amounts of endonuclease in the exonuclease preparation[19,20]. About 10% of circular single-stranded bacteriophage ΦX174 DNA was degraded to mononucleotides by the enzyme in 60 min (1 μg ml^{-1} ^{32}P-labelled ΦX DNA + 100 μg ml^{-1} denatured salmon sperm DNA + 33 μg ml^{-1} enzyme; other conditions as in Fig. 1). The 3T6 cell DNA preparations were not contaminated by endonuclease activity as the rate of digestion by spleen exonuclease of phosphatase- or kinase-treated DNA (Fig. 1) was unchanged by preincubation of the DNA for 8 h at 37 °C or for 1 h at 45 °C (data not shown). Other experiments

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Table 1 Per cent digestion by spleen exonuclease of pulse-labelled and pulse-chase-labelled DNA after alkali or phosphatase treatment

DNA	Size class	Treatment before spleen exonuclease: None	Alkali	Phosphatase
^{3}H-Pulse	*a*	9.5	25	50
^{3}H-Pulse-chase	*a*	5.0	5.0	38
^{32}P-Uniformly labelled	*a*	8.8±0.3	4.7±0.2	43±0.3
^{3}H-Pulse	*b*	8.8	23	53
^{3}H-Pulse-chase	*b*	4.5	4.3	43
^{32}P-Uniformly labelled	*b*	5.0±0.5	3.7±0.2	44±1
^{3}H-Pulse	*c*	18	36	78
^{3}H-Pulse-chase	*c*	8.5	5.8	75
^{32}P-Uniformly labelled	*c*	7.3±0.2	5.0±0.8	69±1

^{3}H-DNA, labelled briefly (pulse) or (see text) for several hours (pulse-chase), was mixed with control ^{32}P-DNA of the appropriate size as indicated in Fig. 2 legend and treated with polynucleotide kinase and ATP in the conditions described in Fig. 1 legend. Each sample was then divided into three portions. One portion was adjusted to 0.4 M NaOH and all three were incubated at 37 °C for 8 h. The alkaline portion was neutralised and one of the two portions not exposed to alkali was treated with phosphatase. DNA in all three samples was extracted with phenol–chloroform as described in Fig. 1 legend and precipitated. All samples were then incubated with spleen exonuclease for 20 min in the conditions described in Fig. 1 legend. Each determination of acid-soluble and acid-insoluble radioactivity involved 5–50×10^3 c.p.m. each of ^{32}P and ^{3}H. The values for digestion of ^{32}P-uniformly labelled DNA accompanying the two ^{3}H-labelled preparations (pulse and pulse-chase) have been averaged and the standard error is indicated.

(not shown) indicated that the extent of digestion of DNA varied with the enzyme concentration, reaching a plateau at 25 μg ml^{-1}. The rate of digestion was tested at different substrate concentrations and was found to be constant in the range 30–200 μg ml^{-1} of DNA. Thus, all digestions with spleen exonuclease were carried out for 20 min at 45 °C with 33 μg ml^{-1} of enzyme and 30–100 μg ml^{-1} of substrate.

DNA synthesised by cultures of 3T6 cells was labelled with ^{3}H-thymidine (^{3}H-dThd) for 1 min at 37 °C after 5 min at 4 °C to allow equilibration of intracellular precursor pools. In some cultures DNA synthesis was abruptly stopped by lysis of the cells with SDS at 100 °C (ref. 10) whereas other cultures were incubated in the presence of unlabelled dThd for an additional 2 h. All lysates were briefly sonicated to fragment the high molecular weight DNA. The preparation was deproteinised by treatment with Pronase and extraction with phenol–chloroform and RNA was separated from DNA by chromatography on nitrocellulose. Sonicated ^{32}P-labelled 3T6 cell DNA was added to the ^{3}H-dThd-labelled preparations to serve as an internal control for all subsequent analyses. The DNA was fractionated into three classes by centrifugation in neutral sucrose density gradients, as shown for the pulse-labelled DNA in Fig. 2. The size of the DNA in pools *a*, *b* and *c* estimated from the sedimentation constants was 4–15, 0.2–4 and <0.2 kilobases respectively. This DNA was then reacted with polynucleotide kinase and enough ATP to give a 500-fold molar excess of ATP over the number of 5′-ends in the sample. Aliquots of the preparation were subjected to alkaline hydrolysis or alkaline phosphatase treatment. The DNA was then tested for spleen exonuclease sensitivity as described above. Immediately before each step in this purification and before all enzyme treatments, the DNA was heated to 100 °C for 20 s. The results of a typical experiment are shown in Table 1.

Treatment of DNA with alkali results in a significantly increased sensitivity to spleen exonuclease digestion in pulse-labelled DNA that is absent in pulse-chase-labelled DNA and in the internal control. We interpret these data to mean that nascent DNA molecules contain ribonucleotides. However, spleen exonuclease sensitivity after alkali treatment alone is indicative of an alkali-labile structure in the molecule, not necessarily a ribonucleotide. The results of four additional experiments on separate preparations of pulse-labelled DNA are summarised in Table 2.

The background levels of spleen exonuclease sensitivity in these experiments are slightly higher than those for the experiment shown in Table 1. The background level of digestion of ^{3}H-pulse-labelled untreated DNA averaged 30%. Treatment with either alkali or ribonuclease before spleen exonuclease increased this to an average of 40%. The greatest increase (18%) was observed with the smallest molecules (size class *c*), the least increase (3%) with the largest molecules (size class *a*). The background digestion of uniformly ^{32}P-labelled DNA averaged 16%. Alkali or ribonuclease treatment increased this average by only 2%. The fact that ribonuclease acts in a manner quantitatively identical to alkali strongly suggests that the increase in sensitivity is due to the presence of ribonucleotides.

Not all the molecules contained ribonucleotides. The maximum extent of spleen exonuclease sensitivity in a 20-min incubation with the enzyme is demonstrated by the samples treated with phosphatase. The resistance to digestion by spleen exonuclease increases with the size of the molecules, probably because the likelihood of the enzyme encountering secondary structure in the DNA also increases. The increase in sensitivity due to alkali or ribonuclease as a proportion of the increase due to phosphatase allows an estimation of the fraction of nascent molecules that possess ribonucleotides. In this manner we estimate from Table 2 that 13%, 27% and 42% of the molecules in size classes *a*, *b* and *c*, respectively, contain ribonucleotides. Thus, the presence of ribonucleotides on the smallest, and presumably most nascent, molecules extends the observations in Table 1 that pulse-labelled, but not pulse-labelled and chased, molecules seemed in part to contain ribonucleotides. Knowing the size of the molecules and assuming that the ^{3}H is uniformly distributed within them, we estimate that 96% of the molecules are in class *c*, so that about 40% of all nascent molecules possess ribonucleotides. This agrees reasonably well with previous studies by other methods on the proportion of Okazaki pieces

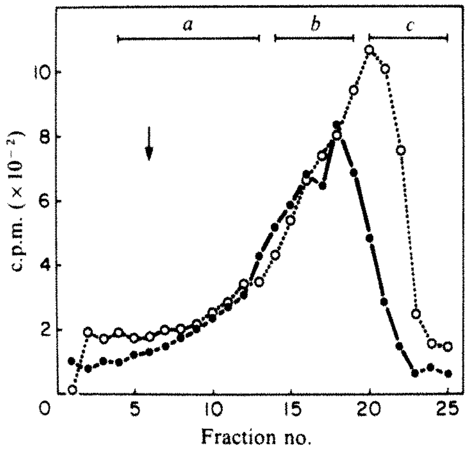

Fig. 2 Neutral sucrose gradient fractionation of ^{3}H-pulse-labelled DNA and uniformly ^{32}P-labelled DNA. 3T6 cells grown to confluence in 5% serum were partially synchronised by replating in 10% serum 24 h before labelling[24]. Cell cultures in plates were floated in an ice bath at 4 °C and exposed to 500 μCi ml^{-1} (60 Ci mmol^{-1}) ^{3}H-dThd in culture medium at 4 °C for 5 min. The plate was then floated in a 37 °C water bath and an equal volume of culture medium at 37 °C containing 500 μCi ml^{-1} ^{3}H-dThd was added to the plate. We find that this method of pre-equilibration with dThd[25] results in the incorporation of two to five times more ^{3}H-dThd during the period at 37 °C. After 1 min at 37 °C DNA replication was rapidly terminated by lysing the cells with 1% SDS at 100 °C for 1 min[10]. The lysates from 2–4×10^7 cells were pooled and sonicated briefly to reduce the size of the high molecular weight DNA. The lysate was then treated with Pronase-B (1 mg ml^{-1}, 37 °C, 2.5 h in the presence of 0.4 μg ml^{-1} polyvinyl sulphate), extracted twice with phenol–chloroform (1:1) and precipitated with isopropanol. Over 90% of the RNA was removed by chromatography on nitrocellulose[10]. Uniformly ^{32}P-labelled DNA was prepared by growing 3T6 cells for 2 d in phosphate-free medium containing 10 μCi ml^{-1} ^{32}P-orthophosphate. It was purified as described above using sonication to reduce its size. Nascent ^{3}H-DNA and total ^{32}P-DNA were fractionated together after brief denaturation on 5–20% sucrose gradients in 1 M NaCl, 1 mM EDTA and 50 mM Tris-Cl, *p*H 8, in a SW56 rotor at 50,000 r.p.m. for 3.5 h at 20 °C. Fractions were collected from the bottom, assayed for radioactivity and pooled as indicated. The arrow denotes the position of single-stranded linear ΦX174 DNA (24S). ○, ^{3}H-pulse-labelled DNA; ●, ^{32}P-total DNA.

Table 2 Per cent digestion by spleen exonuclease of pulse-labelled DNA after alkali, ribonuclease or phosphatase treatment

DNA	Size class	Treatment before spleen exonuclease			
		None	Alkali	Ribonuclease	Phosphatase
^{3}H-DNA (nascent)	*a*	35	37	39	59
	b	28±3	36±3	38±3	61
	c	28±4	46±2	46±2	71±9
^{32}P-DNA (sonicated uniformly labelled)	*a*	24	23	21	60
	b	15±2	19±4	20±3	59
	c	10±3	12±5	16±3	69±9

Cultures of 3T6 cells were pulse-labelled with ^{3}H-dThd as described for Fig. 2. After treatment with Pronase for 1 h the lysate was heated at 100 °C for 4 min, chilled and centrifuged for 45 min at 45,000 r.p.m. in a Ti60 rotor at 4 °C to separate high and low molecular weight DNA. The supernatant contains 0.1% of the total DNA and 75–80% of the incorporated radioactivity[10]. DNA in the supernatant was then purified as described for Fig. 2 without sonication, mixed with sonicated ^{32}P-DNA and fractionated into the three size classes on neutral sucrose gradients. Further analyses were carried out as described for Table 1. Ribonuclease treatment used ribonuclease A (Worthington), 20 μg ml^{-1}, plus ribonuclease T1 (Worthington), 20 units ml^{-1} (heated at 80 °C for 10 min to inactivate DNases), for 30 min at 37 °C. Each determination of acid-soluble and acid-insoluble radioactivity involved $5\text{–}50\times10^2$ c.p.m. each of ^{32}P and ^{3}H. The values given for the *b* and *c* size classes are the average of four experiments on four separate preparations of ^{3}H and ^{32}P DNA; the s.e.m. is indicated. When no standard error is shown only one experiment was carried out. Degradation of control 5′-phosphorylated DNA may result from contaminating endonucleolytic activity[19,20]; our observations that 10–15% of circular ΦX DNA is degraded support this possibility. The enzyme concentration, size and type of DNA, conditions of digestion, and techniques used to measure hydrolysis affect the results. In some of our experiments, especially this Table, 5′-phosphorylated DNA was degraded more than the usual 10–15%. We think this is because the ^{32}P-labelled DNA was a few weeks old and that radiolysis had enhanced its sensitivity to alkali or enzymes. The data in Table 1 were obtained with freshly prepared ^{32}P-DNA. The ^{3}H-DNA was always used as soon as practicable after its preparation and we have no reason to think that radiolysis was contributing significantly to its sensitivity to spleen exonuclease. We have found that fragmentation of the DNA by either sonication or partial digestion with DNase I yielded preparations which showed similar low (or high, after phosphatase treatment) sensitivities to spleen exonuclease.

possessing oligoribonucleotide primers which place the value at 33–50% (refs 8, 21).

We routinely observed that nascent DNA not treated with alkali or RNase was more sensitive to spleen exonuclease digestion than was non-nascent DNA, particularly among the smallest molecules after correction for the degradation of the control ^{32}P-DNA. This might be due to the presence of structures in some nascent molecules that are sensitive to an endonuclease activity in the exonuclease preparation (for example, apyrimidinic sites of uracil excision).

The existence of RNA priming of DNA synthesis has been regarded with caution because of the difficulty in demonstrating this mechanism *in vivo*[22]. This is especially true of mammalian DNA, where such data have been lacking. The above experiments show that it is possible to detect this mechanism *in vivo* in intact mammalian cells by using different methods from those used in the analysis of the products of synthesis *in vitro*.

While this work was undergoing review Tseng *et al.*[23] reported results showing good evidence for the presence of short oligoribonucleotides on the 5′-ends of nascent DNA molecules synthesised *in vivo* in human lymphoblasts.

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Biological degradation of TCDD in rats

H. Poiger & Ch. Schlatter

Institute of Toxicology, Federal Institute of Technology and University of Zurich, Zurich, Switzerland

Apart from its high toxicity[1,2], the chemical stability and persistence of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in the environment bring about additional hazards[3,4]. However, until now there has been no evidence for degradation of this dioxin in biological systems, and it is considered unusual that a compound which is insusceptible to biological attack should be so extremely toxic. In *in vitro* studies[5] with microsomal preparations from mouse, rat and rabbit liver, as well as *in vivo*, no metabolite of TCDD has been detected by several workers[6–8]. Recent data, showing a lower toxicity of TCDD in rats after stimulation of the hepatic mixed-function oxidase, suggested possible metabolic transformation of this compound[9]. In the present study, we used tritiated TCDD to search for metabolites in the bile of rats and found marked changes in lipophilicity and mobility in TLC of the excreted radioactivity. Treatment with diazomethane yielded at least two new compounds. These observations hinted at the formation of phenolic hydroxyl groups in the dioxin molecule.

Commercially available tritium-labelled TCDD with a specific activity of 41 Ci mmol^{-1} was purified by preparative gaschromatography using consecutively two stationary phases with different polarity (OV 101, DC 560); a radiochemical purity higher than 98.7% was thus obtained. The test animals were female rats from the Sprague–Dawley-derived ZUR:SIV-Z strain, weighing about 260 g. The bile duct was cannulated in both directions with polyethylene catheters which then led to the neck of the animal. After a recovery period of a few days the rats received oral doses of about 20 μCi TCDD dissolved in corn oil and were placed in suitable restriction cages allowing free access to water and feed. Bile was collected in a fraction collector for 3–4 days and then the animals were killed.

The concentration of radioactivity in the bile remained relatively constant throughout the whole experimental period, about 1% of the dose being excreted daily. In the liver tissue, 8.5–12% of the administered radioactivity was found. This radioactivity could be extracted almost completely using dichloromethane as solvent, with about 2% remaining in the residue. In contrast, only 1.5% of the radioactivity from the bile was extractable. This radioactivity was dialysable, so that binding to proteins or other macromolecules could be excluded. However, after incubation of the bile (or the dialysate) with glucuronidase/arylsulphatase (Boehringer), 75% of the radioactivity moved into the organic phase. Only a small part could be removed by lyophilisation (5% from bile, 0.2% from liver). This fraction most probably represents tritiated water because the specific activity remained constant in several successive fractions of the distillate and was not extractable with hexane.

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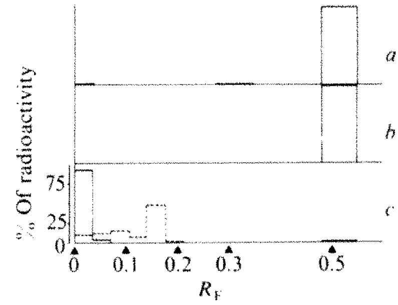

Fig. 1 Radio-TLC of: *a*, tritiated TCDD (as received, not purified); *b*, liver extract; *c*, bile extract (after enzymatic treatment). Precoated silica-gel 60 sheets (Merck), with dichloromethane as developing solvent, were used. The dotted lines show distribution of radioactivity after methylation with diazomethane. Levels of radioactivity were determined in scrapings of 0.5–1 cm zones and are expressed as per cent of the total amount of radioactivity on the silica-gel sheet.

In TLC, radioactivity extracted from the liver was localised at an R_F value of 0.52, where TCDD was also found (Fig. 1). The compound extracted from bile after the enzymatic treatment behaved quite differently. Most of the activity was concentrated at the starting zone, demonstrating a more polar character, with only a small fraction, 1.5%, located at R_F 0.55. Gaschromatographic examination of this extract on a SE 30 column revealed a retention time of the radioactivity similar to that of TCDD; TLC mobility of the material trapped during the GLC examination remained unchanged. When labelled TCDD was added to bile and the mixture left to stand for a few hours, this added fraction could always be extracted with dichloromethane and was found at the position of TCDD on thin layer chromatograms. Thus, chelation of TCDD by any constituents of the bile was excluded. Treatment of bile extracts with diazomethane led to the formation of at least two new compounds with lower polarity than TCDD, exhibiting R_F values of about 0.09 and 0.16.

As only small amounts of the compound were available—concentration in the bile fluid was 50–100 pg ml^{-1}—attempts at mass spectrum analysis were unsuccessful.

Thus, the present results indicate that TCDD is excreted into bile in a metabolised form, as a water-soluble conjugate. These conjugates can be cleaved by enzymatic treatment yielding relatively polar compounds with low mobility on thin layer chromatograms. Esterification with diazomethane diminishes polarity and consequently mobility of the derivative on TLC is increased. Most probably, conjugation occurs with phenolic hydroxyl groups, but their position and number are unknown. Preliminary experiments with double-labelled $^{14}C/^{3}H$-TCDD showed no change of the ratio of the two isotopes after metabolism. This observation makes a hydroxylation at the *ortho* positions unlikely. However, cleavage of the ether group also seems feasible. A decision between these possibilities cannot yet be made.

^{3}H-TCDD was obtained from A. Kende, Rochester, New York.

Note added in proof: Similar results have recently been reported[10].

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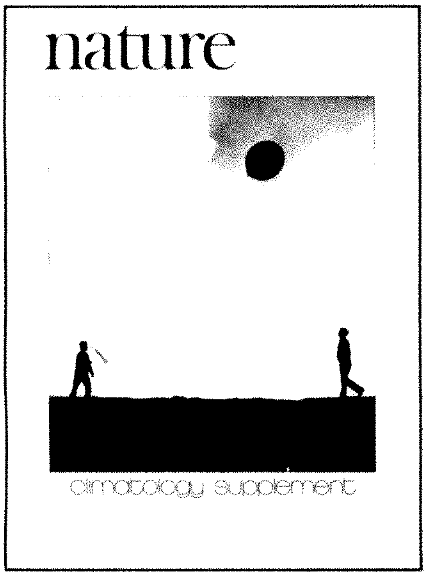

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matters arising

Interstellar grains: organic or refractory?

IT has become fashionable to discuss the possibility that interstellar grains may contain significant quantities of organic material (see ref. 1 and refs therein). I draw attention here to new data which preclude the presence of most organic solids as a significant component of the grains in two typical dark clouds. As pointed out by Duley and Williams[2], virtually all organic compounds containing C and H possess an infrared absorption feature due to C—H stretching at 3.3–3.4 μm. The spectra of several dust-embedded stars in the Ophiuchus and Corona Austrina dark clouds were recently observed with the Anglo-Australian Telescope (Whittet and Blades[3]), in a programme primarily aimed at studying the 3.1 μm ice band. None of these sources, whose visual absorption falls in the range 5–20 mag. shows a feature at 3.3–3.4 μm: the detection limit is an order of magnitude less than the optical depth predicted by Fig. 1 of ref. 2. Similarly (as noted in ref. 2) the spectra of molecular cloud sourcesswith deep ice bands observed by Merrill *et al.*[3] show no C—H feature. It must therefore be concluded that organic grains cannot constitute a significant part of interstellar grain material, even in dense clouds where from other considerations, grain growth is known to occur[5,6].

It seems that the only model for interstellar grains which can simultaneously satisfy their well established dielectric properties[3,7], cosmic abundance constraints[8], and the infrared spectra of highly reddened stars[3,4,9], is one involving a mixture of refractory particles involving compounds of oxygen—silicates and metal oxides—with a possible addition of water ice in the densest regions. Most of the carbon in interstellar clouds is likely to be tied up in gas-phase CO. Candidate grain materials, such as SiO_2, FeO and MgO, are, indeed, abundant in the carbonaceous chomdrites[10].

D. C. B. WHITTET

Department of Physics and Astronomy,
University College London,
Mill Hill Park,
London NW7, UK

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SAGAN AND KHARE REPLY—We have proposed[1] that a complex organic material, called tholins, produced by electrical discharge or ultraviolet (UV) light from mixtures of cosmically abundant gases, is an important candidate material for the interstellar grains (although other material such as ices and silicates must, of course, be present); and that the sputtering and spallation products of tholins can explain the gas phase organic molecules discovered in the interstellar medium by microwave line spectroscopy. As noted in Fig. 3 of our paper[1] and elsewhere[2], little or no IR absorption is evident in the interstellar medium in the 3.3–3.4 μm region, characteristic of the C—H vibrational transitions of many organic molecules. The observational upper limits correspond, for example, to 10% of carbon in interstellar grains in alkenes and aromatics and 1% in alkanes (ref. 2), and an order of magnitude less according to Whittet. However, as clearly indicated in Table 1 of our paper[1], the atomic abundance of hydrogen by number in tholins is only a few per cent; and, as is apparent from Fig. 2 of our paper, in most laboratory tholins there is no trace of absorption features in the 3.3–3.5 μm range. We have re-examined various IR spectra of tholins and find no clear C—H stretch peak for raw UV tholins; for UV tholins with elemental sulphur removed; for UV tholins with elemental sulphur removed and the sample heated to 450 °C; for spark tholins; and for spark tholins heated to 650 °C. Tholins have a significantly different structure from graphite. We also re-emphasise that a range of other IR absorption features, reliably identified in the interstellar medium, cannot be understood by refractory materials and ices alone; but have a natural explanation if tholins are a constituent of the interstellar grains. The missing ingredient in the interstellar medium seems to be a complex organic solid which has a very weak C—H absorption feature; tholins seem to satisfy these requirements.

CARL SAGAN
B. N. KHARE

Laboratory for Planetary Studies,
Cornell University,
Ithaca, New York 14853

1. Sagan, C. & Khare, B. N. *Nature* **277**, 102–107 (1979).
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The maximum entropy method

THE exposition of the maximum entropy method (MEM) given by Gull and Daniell[1] has been strongly criticised[2] on the basis that it led to some definite solution in situations in which different types of solution are possible (for example, one-peak (one source in astronomical language) and two-peak (double source) solutions). Here we defend Gull and Daniell's theory.

No matter how precise the measurements are, for sufficiently small δ, a point source gives the same observational information as do two nearby point sources at distance δ. Therefore among all brightness distributions, I, that are in accordance with observations, there are values of I corresponding to many-component sources (with as many components as one wishes) with very complicated structure. Astrophysicists want the distribution that contains, crudely speaking, the minimum possible number of components, or, more precisely, the simplest possible distribution in some formal sense. We show here that MEM solves this problem satisfactorily.

How can we formalise this demand? Assuming that complexity is a functional $\int s(I)$. We suppose that it can be represented in the form

$$s_0 + \int s_1(\bar{\sigma}, I\bar{\sigma}))\,\mathrm{d}\bar{\sigma} + \int s_2(\bar{\sigma}_1, \bar{\sigma}_2, I(\bar{\sigma}_2), I(\bar{\sigma}_2))\,\mathrm{d}\bar{\sigma}_1\,\mathrm{d}\bar{\sigma}_2 + \cdots$$

(any analytic functional can be represented in such form). Axiomes for S are as follows. (1) Many-component source. Assume

$$I = I_1 + I_2,\ \mathrm{supp}\ I_1 n\ \mathrm{supp}\ I_2 = \phi,\ \mathrm{supp}\ I_1 = \mathrm{supp}\ I_{1'}$$

We demand that

$$S(I_1) < S(I_{1'}) \rightleftarrows S(I_1 + I_2) < S(I_{1'} + I_2)$$

(if one component is fixed, the image is simpler if the second is simpler). (2) Translational invariance. For any translation T: $S(I_1) < S(I_2) \rightleftarrows S(TI_1) < S(TI_2)$. (3) Independence on choice of unit for flux I: if $S(I_1) > S(I_2)$ and I_1, I_2 are consistent with the same observations (that is

$$\int I_j(\bar{\sigma}) \int A_i(\bar{\sigma}) = C_i \text{ for } j = 1, 2$$

and some fixed $C_i, A_i, A_0 = 1$), then $S(\lambda I_1) > S(\lambda I_2)$.

In looking for the simplest values of I an additive constant in S is inessential. Assuming $S_1 = S_2$ if $S_1 - S_2 = \text{const.}$, then one can prove the following. If S satisfies

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points (1)–(3), then either

$$S(I) \sim K \int I \ln I \, \mathrm{d}\bar{\sigma},$$

or

$$S(I) \sim K \int I^{\alpha} \, \mathrm{d}\bar{\sigma}$$

for some constants K, α ((1) leads to $S_2 = S_3 = 0$, (2) leads to

$$S(I) \sim \int \tilde{\bar{S}}_1(I(\bar{\sigma})) \, \mathrm{d}\bar{\sigma}$$

for some $\tilde{\bar{S}}_1$).

MEM is usually chosen, because in all other cases $I \geqslant 0$ is an extra complication and here it follows automatically from the variational principle.

V. JA. KREINOVIC
O. M. KOSHELEVA

USSR 196140 Leningrad 140,
Pulkovo Special,
Astrophysical Observatory

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FIDDY AND GREENAWAY REPLY—We thank Kreĭnovič and Kosheleva for their remarks concerning our criticism[1] of phaseless object reconstruction using the maximum entropy algorithm[2] and other statistical techniques. The paper giving a more detailed explanation of our criticism is not widely available[3]. Because of this, we are taking the opportunity to supplement our necessarily brief discussion[1], and to comment on the points which they have raised.

Their statement in the paragraph beginning "No matter how precise . . ." is an oversimplification. The claim may be true if the multiple sources in question are distributed over a region unresolved by the measurement baseline. However, if the measurements are precise, one may analytically continue the data and thus amend the resolution criteria. Also, the use of entire function theory enables one to predict a countable, complete and often finite set of possible solutions, from precise measurements. In ref. 3 we give examples of the possible solutions consistent with an intensity distribution. In only one case do all the solutions correspond to a double source. The essential point, which is apparent from the other examples, is that even within the given resolution criteria for the truncated data, there is the danger of confusing single and multiple sources when making phaseless reconstructions.

To say that one only requires "the distribution that contains . . . the minimum possible number of components", implies lack of interest in multiple systems. If only upper limits on source extent are required, one may use the measured object autocorrelation. If the source structure is of interest, it is important to ask what the ambiguities are, and to classify them as possible single, double, triple systems. Entire function theory predicts that such a classification is possible, the range of solutions being countable.

We do not deny the usefulness of the maximum entropy method, since with phase information it provides an extremely powerful tool. Gull and Daniell[4] note the possible existence of several entropy maxima and comment that in such cases the reconstruction "will be misleading". They continue "with phase information . . . this cannot happen". We concur, believing that such multiple entropy maxima correspond to ambiguities of the type discussed in refs 1 and 3.

M. A. FIDDY
A. H. GREENAWAY

Physics Department,
Queen Elizabeth College,
Campden Hill Road,
Kensington, London W8, UK

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Effects of sodium butyrate on human chronic myelogenous leukaemia cell line K562

ANDERSSON and his colleagues have postulated that the K562 cell line is of erythroleukaemic origin[1] and can be induced to undergo erythrocytic differentiation by sodium butyrate[2]. However, treatment with sodium butyrate of the original K562 cells fails to produce benzidine-positive staining, cell haemoglobin crystals or the synthesis of human globin.

The K562 cells (initial density 2×10^5 cells ml^{-1}) were cultivated in medium described elsewhere[3–6] in the presence or absence of 1 mM sodium butyrate. In contrast to Andersson's observation, sodium butyrate inhibited the cell growth by 50%. Thus, after 3 d, control cultures contained 8.7×10^5 cells while butyrate-treated cultures had 4.3×10^5 cells. No differentiation was detectable. The Lepehne reaction did not reveal a single benzidine-positive cell. We did, however, observe that cells from cultures treated with butyrate showed various signs of degeneration such as vacuolation, hyaline degeneration and loss of the cytoplasmic basophilia. Electron microscopy also showed various signs of cell injury.

In the normal sequence of erythroid differentiation the cell progresses through a series of definitive recognisable stages culminating in a mature erythrocyte. Andersson *et al.*[2] showed the intracytoplasmic generation of erythrocyte-like bodies which were subsequently expelled from the original cell. This phenomenon has not been described before and these 'erythrocyte-like' particles are probably only hyaline bodies and/or pieces of cytoplasmic debris. Furthermore, neither treated nor control cells synthesised α- or β-globin chains (Fig. 1) as would be expected if erythroid differentiation has occurred. With the report of Rutherford *et al.*[7] in mind, note that embryonic globin polypeptides are not detectable by the column chromatographic procedure used. Thus, it is not possible to exclude the possibility of synthesis of trace amounts of embryonic globin polypeptides, but it seems convincing that adult globin was not synthesised in cells treated with butyrate.

Because our K562 cells do not stain positively after exposure to sodium butyrate we also studied the effect of sodium butyrate in myelosarcomas made up of K562 cells transplanted in nude mice[8,9]. Six intraperitoneal injections of sodium butyrate (275 mg per kg every 2 d) produced extensive necrosis of the myelosarcoma. Imprints from K562 tumours growing *in vivo* in nude mice also failed to show benzidine-positive cells. However, when we attempted to reculture the cells from tumours, ~10–15% of the cells did give positive benzidine staining. These cells were not typical erythroid cells and showed well defined nucleoli. It is most likely that they were of mouse origin or the K562 cells were somehow altered by passage in the mouse to give a false positive benzidine reaction. If Andersson's cells have at any time been passaged in immunodeficient mice, this may account for the benzidine-positive cells. We do not know which clone or subline of K562 was used by Andersson *et al.*[1]. In March 1978, we sent to Dr Eva Klein a clone of K562 which was recultured *in vitro* after being serially transplanted in nude mice for 20 passages. Klein also had a subline of K562, originally provided to Dr G. Henle six years ago, and the myeloid origin of this subline was confirmed by Klein *et al.*[10]. Evidence for the myeloid nature of K562 cells is further supported by the presence of group-specific granulocyte antigens[11].

Another claim of erythroid origin rests primarily on the biosynthesis of glycophorin A, the major sialoglycoprotein of human erythrocytes, by K562 cells[12]. Unpublished results show a very low number of glycophorin A cells in our K562 cell line and no conclusions can be made yet.

In summary, the failure of sodium butyrate to induce erythroid differentation of K562 cells is consistent with our earlier studies on the effects of dimethyl sulphoxide[13]. Both agents produce cytotoxic effects rather than a stimulation of the cell potential for differentiation. The discrepancies between our findings lead us

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to question whether Andersson's cells are indeed K562 cells. The K562 cell line was originally established in our laboratory from a pleural effusion of a patient with chronic myelogenous leukaemia in terminal blastic crisis[3-5] and has been found to be a highly undifferentiated cell of the granulocytic series[5,6,10,11] with a consistent ultrastructural pattern[14]. It is the progenitor of all K562 sublines now in existence. The possibility of spontaneous changes through clonal evolution or of contamination with other cell lines and subsequent overgrowth or hybridisation cannot be discounted after the cells have been grown outside our laboratory for so many years. Characteristics lacking in the parent cell line may be present in a subline which acquired them at some stage after leaving our laboratory. In any event, the classification of the K562 cell line as an erythrocytic precursor is certainly premature for the evidence available. The presence or absence of glycophorin A in highly undifferentiated precursors such as the stem and differentiating cells has not yet been established and so it should not be considered solely as a marker of the erythrocytic series. Morphologically, the K562 blasts are probably closer to haemocytoblasts than pronormoblasts or proerythroblasts depending on the terminology used.

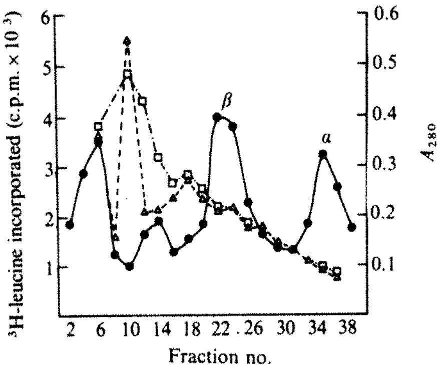

Fig. 1 Elution profile of globin chains on CM-cellulose-urea columns. Absorbance of globin chains from carrier human haemoglobin (●) is shown in comparison to the incorporation of ^{3}H-leucine into polypeptides obtained from control cells (□) and from K562 cells incubated in the presence of 1 mM sodium butyrate (△). After addition of label at 48 h cells were pelleted at 12 h of incubation and then washed in excess saline[15]. H_2O (1 ml) and carrier human haemoglobin was added. Globin was prepared by precipitation in acid acetone (1.2% HCl in acetone)[16]. The lyophilised globin was resolved into constituent polypeptides by chromatography on CM-cellulose columns in 8 M urea[17]. A phosphate gradient from 0.005 M to 0.03 M was used and 2.5-ml fractions collected. Aliquots (0.5 ml) were then used for scintillation counting.

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CARMEN B. LOZZIO
BISMARK B. LOZZIO
EMILIO A. MACHADO
JOSEPH E. FUHR
STEPHEN V. LAIR
ELENA G. BAMBERGER
University of Tennessee,
Department of Medical Biology,
Memorial Research Center,
Center for Health Sciences,
Knoxville, Tennessee 37920

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ANDERSSON *ET AL.* REPLY—Lozzio *et al.* challenge our conclusion that the K562 cell line has the capacity for erythroid differentiation and raise doubts that we have studied the authentic K562 line.

The cell line was originally obtained from Lozzio via W. Henle. A detailed account of its characteristics was given by Klein *et al.*[1]. The karyotype of our K562 cells is obviously identical to that originally described by Lozzio and Lozzio[2]. Contamination with mouse cells or with mouse/human hybrids can be excluded, as the cells have never been carried *in vivo.*

Concerning the erythroid versus granulocytic nature of the K562 cells, we have demonstrated that: (1) The surface glycoprotein pattern of K562 cells resembles that of normal erythrocytes[3] but is completely different from those of other haematopoietic cell lines[4,5] of the promyelocytic leukaemia line HL-60[6] and of granulocytes[7] and their benign and malignant precursors[8]. (2) Our K562 cells carry on their surface 1.1×10^6 glycophorin A molecules per cell[9] which are synthesised by the cells[10]. (Glycophorin A is the major sialoglycoprotein of human red cell membrane and is present only on erythroid cells in human bone marrow[11].) (3) K562 cells synthesise haemoglobin in the presence of sodium butyrate[12] as shown by the Lephene's benzidine and by radioimmunoassay indicating the presence of both the haem and the globin parts. Rutherford *et al.* recently reported that K562 cells synthesise embryonic and fetal haemoglobin in the presence of haemin[13]. The identities of the haemoglobins have recently been confirmed by peptide mapping (T. R. Rutherford, personal communication).

The different observations on phenotype of the K562 cells by us[12] and Rutherford *et al.*[13], as compared to those by Lozzio and Lozzio[2], are not easily explained. As the original K562 cell line was not initially cloned the possibility exists that we have been investigating selected subpopulations of the cell line endowed with different differentiation capacities. (This is also frequently seen in the Friend leukaemia system.) Therefore we suggest an interchange of our respective K562 cells for comparative studies.

Regardless of the real phenotype of the K562 cells, the establishment of this unique cell line by Lozzio *et al.* is highly creditable. This has provided an excellent model for human haematopoietic differentiation.

Note added in proof: Recently we obtained K562 cells (passage 239) directly from Lozzios' laboratory (courtesy of Dr R. A. Laine). These K562 cells have a strong surface expression of glycophorin A as shown by immunofluorescence and immunoprecipitation from surface labelled cells with specific antiglycophorin A antiserum.

LEIF C. ANDERSSON
Transplantation Laboratory,
and Department of Pathology,
University of Helsinki

MIKKO JOKINEN
CARL G. GAHMBERG
Department of Bacteriology and Immunology,
University of Helsinki,
Helsinki, Finland

EVA KLEIN
GEORGE KLEIN
Department of Tumor Biology,
Karolinska Institutet,
Stockholm, Sweden

KENNETH NILSSON
The Wallenberg Laboratory,
University of Uppsala,
Uppsala, Sweden

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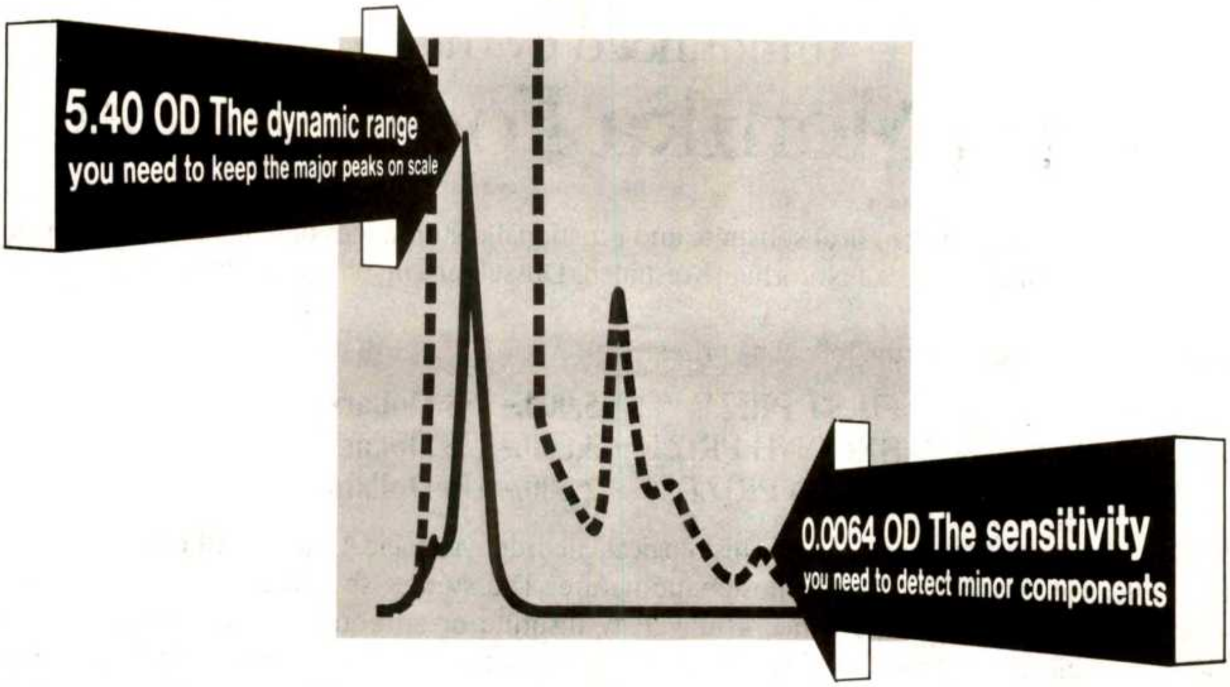
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reviews

Management of controversy

Eric Ashby

Controversy: Politics of Technical Decisions. Edited by Dorothy Nelkin. Pp. 256. (Sage Publications: Beverly Hills and London, 1979.) Hardback $16, £10; paperback $7.95, £5.

SCIENCE-AND-SOCIETY, hyphenated like this, is now a well established subject for study, with departments, professors, journals, conferences, research grants: all the apparatus of an academic discipline except a codified content. This is not a pejorative assertion. A codified content takes time to evolve; premature generalisations might become obstacles to evolution; the most helpful contributions to make at this stage are in the 'natural history' of the subject: descriptions of what actually happens (the echo of Ranke's recipe for writing history "*wie es eigentlich gewesen*") and cautious comparisons of what seem to be similar episodes.

This is what Dorothy Nelkin has been doing for some time. She has published case-studies of controversies about the siting of a nuclear power station, about the extension of the Boston airport, about housing politics, about military research at MIT; all controversies: for the first lesson the novice has to learn in this subject is that sweetness-and-light is not only impracticable, it is undesirable. Controversy is essential to the resolution of the problems that arise from the applications of technology to social ends. It's a consequence of political freedom. So attention has to be given, not to the elimination of controversy but to its management. Who has the right to take part? How can those who take part have access to information? What is the appropriate forum for the controversy: a court of law, a ministerial public enquiry, a panel of experts, a clash of pressure groups? Experiments which might provide answers to these questions are going on all the time. The Royal Commission on Electric Power Planning in Ontario has published some notable material; so has the Electric Power Research Institute in California; so, in Britain, has the Advisory Committee on Trunk Road Assessment. But the most useful material for the student is a collection of documented case-studies, as little coloured by prejudice as is possible in a subject where exaggeration, sensationalism, and distortion of the facts are the very raw material in the process.

This is what Dorothy Nelkin has achieved in her editorship of this useful collection of twelve case-studies in the politics of technical decisions. The studies are intended to "provide students with a sense of the kind of reasoning that motivates public agencies, government officials, scientists, and protest groups". She distinguishes four kinds of controversy and the contributors to the volume give three examples of each kind. They are: efficiency versus equity, as in the siting of an airport; benefits versus risks, as in the controversy over nuclear wastes; regulation versus freedom of choice, as in the prescription of certain drugs not because they are dangerous but because they appear to be useless; and science (the rational approach to nature) versus traditional values (opposition to the pursuit of knowledge irrespective of where it might lead.) Each case-study is well supported by references to primary sources.

I would like someone to write a monograph on controversies about airports. Heathrow is a shameful example of the incompetence of the British in the planning of air transport and the futility of the present state-of-the-art of technology assessment. But we have no monopoly of incompetence. In the 1950s there was a vast investment in Gander airport in Newfoundland, "just before the introduction of jet aircraft made nonstop trans-Atlantic flights possible". Then, as a case-study of the Toronto airport goes on to explain, came the proposal to site a second airport in Toronto. One motive was rivalry with Montreal; if Toronto didn't expand, Montreal would become the "gateway to Canada". Another motive was to pacify people living near the present airport. Opposition to a new airport developed rapidly, just as it is now doing in Britain to oppose any site for a third London airport. It coalesced under a good emotive label: POP — People or Planes. Among the supporters of the opposition was one aircraft manufacturer, who saw a better market for short take-off craft if there were to be only one airport in Toronto. One interesting and significant point which the essay makes is that the real issues were political but they were often camouflaged as scientific or technical.

The most interesting essay in the book, because it draws upon almost inaccessible sources, is one on environmental conflict in the USSR. With a wealth of references, almost all Russian, Thane Gustafson describes the awakening of an environmental conscience in the Soviet Union. He effectively dispels any notion that it came from the grassroots. But he shows that the genuine concern about the condition of Lake Baikal was not solely for commercial expediency: "Through literature and folklore, every Russian from childhood knows its beauty". And this was a powerful factor in the campaign to clean the lake. Concern for water management and opposition to the siting of dams arose because it was causing a serious alienation of valuable agricultural land. But the new environmental programme owes its impetus to the influence of scientists, aware of the concern for conservation in Europe and America, who have made themselves audible in the councils of the Kremlin.

All the case-studies are interesting and a few comments on them will illustrate how well they have been chosen. The familiar ban on the use of diethylstilbestrol (DES) is an example of the need to balance the benefits of more meat against the potential disbenefits of cancer: one a certainty, the other an unproven possibility. It is noteworthy that the Food and Drug Administration avoided justifying the ban on grounds of the notorious Delaney Clause because President Nixon proposed to Congress that this clause should be "diluted" to avoid some of the more crazy consequences of its application "to ban all foods because they contain a few molecules of naturally occurring carcinogens".

There is a historical chapter, showing that controversies about the uses of science are nothing new, which traces the opposition to smallpox immunisation in the eighteenth and nineteenth centuries. And there is an ominous account of an attempt — yes, as late as the 1970s — to give equal treatment to the theories of evolution and of special creation in the teaching of biology in California. California buys about 10% of the nation's textbooks and it was seriously proposed that special creationists should have at least equal consideration in textbooks with the consideration given to orthodox biology. The National Academy and the American Association for the Advancement of Science had to throw their weight into the controversy. Perhaps we should not be surprised; astrologists can still make a good living in Britain and it is often said that more people make a living out of astrology than out of astronomy.

This book is a useful addition to a small, but growing, literature of case-studies of this kind. By far the best, though its scope is limited to planning enquiries, is Roy Gregory's *The Price of Amenity* (Macmillan, 1971). There is also, for British examples, the book edited by Peter Smith, *The Politics of Physical Resources* (Penguin, 1975), though some of the essays in this book are far from objective; and a book edited by R. Kimber and J.P. Richardson, *Campaigning for the Environment* (Routledge and Kegan Paul, 1974). The most comprehensive summary, though the case-studies are unfortunately very brief, is in the book edited by Edward Lawless, published by Rutgers University Press in 1977 (reviewed in *Nature*, **274**, 295-296; 1978) under the unpromising title *Technology and Social Shock*.

So there is now the nucleus of a library which will promote the evolution of science-and-society into a respectable discipline. Dorothy Nelkin's latest contribution to this is very welcome. ☐

Lord Ashby is Chancellor of Queen's University, Belfast, and Fellow of Clare College, Cambridge, UK.

Encyclopaedic approach to technology

A History of Technology. Vols VI and VII: *The Twentieth Century, c.1900 to c.1950.* Edited by T.I. Williams. (Clarendon/Oxford University Press: Oxford, UK, 1979.) £25 and £27.

TWENTY-FIVE years ago the now classic first volume of *A History of Technology* appeared from the Clarendon Press. This year, we welcome the sixth and seventh (and last) volumes in the series. With them we arrive, in history, where the series itself began, in the early 1950s. Together, these seven magnificent volumes form a monument of scholarship. Monuments normally inspire criticism or veneration; rarely use. This one is different.

From the early days of his collaboration with Charles Singer, E.J. Holmyard and A.R. Hall, Williams enjoyed the cooperation of a legion of eminent scholars whose unquestioned technical expertise made their contributions definitive. In editorial standards, range and clarity, the present volumes continue their predecessors' tradition of high scholarship. Nonetheless, 25 years is a long time in historiography, as in technology. Moreover, a new generation (and profession) of historians has emerged, with significantly altered perceptions of the social function of technology and technologists. With them, the reader is compelled to ask in these more cautious times, has the history of technology progressed as far as the technology it describes?

In reply, these two volumes invite comment at three levels. First, these chapters are, in technical terms, proverbially encyclopaedic in breadth and detail. Like their predecessors, these volumes describe a spectrum of activity, from fuels to food, metals to textiles, chemicals to buildings and transportation. Some chapters bring the story 'up to date'; others (space, aircraft, medical and ocean technology, and computers) describe recent additions to the "relief of man's estate". Although experts may differ (a possibility frequently raised, if indifferently addressed), one can scarcely debate the pronouncements of Lord Montagu of Beaulieu on motor cars, or of Colin Buchanan on town planning. Several other chapters — including Frank Greenaway's on instruments and Brian Bowers' on electricity — offer fresh insights as well as information. But these technical chapters together seem less than the sum of their parts. There is, despite the editor's catholicity, a deceptive provincialism of outlook which emerges in many of them. A mixture of "practical men" and university men (in about equal parts) is not necessarily an unsatisfactory formula for a fine product; but the fact that the contributors are overwhelmingly British, and, with perhaps ten exceptions, are not professional economic or social historians, points clearly to one difficulty. Another lies in the subject-matter itself.

One cannot criticise the authors for failing to make obligatory reference to microelectronics or biotechnology (although, indeed, there are none). But it will not escape notice that many of the 58 contributions (notably excepting the editor's own) follow historiographical conventions remarkably similar to those of educated amateurs a generation ago. Most technical chapters are history preserved in aspic, sadly neglecting systematic comparative analytical discussion. Excellent descriptive accounts of leading technical developments in different fields may nonetheless strike many a current reader, of say, "Technology and Culture", as dry, stylised and lacking in economic and social insight. On the one hand, few of the technical chapters even attempt to demonstrate the connection between entrepreneurial and management styles in research and development, and the problems of marketing and diffusion in different countries under different economic systems. On the other hand, few contributors pause to ask, how can we use this knowledge to understand the developments of the *next* twenty years?

Second, as the editor explains, technology advances across a broad front, or not at all. Yet the editorial arrangement of technical contributions, inevitably dividing knowledge into arbitrary categories, militates against the authors asking larger questions about the nature of the technologies concerned. Today, whatever may be one's opinion, for example, of 'appropriate technology', the reader will want to know in what ways technological development (whether in fuel, transport, housing, and so on), has been shown capable of responding to altered economic and social realities. It could be argued, in defence, that in the 1950s, most scholars and the English reading public were as uninterested in the relationships of production as they were unaware of the implicit risks of technological development. But whatever one's approach to economic history, no account of technological development which omits to describe the organisation and operation of the modes of production characteristic of industrial capitalism can today be considered complete.

If this criticism is sound, it is even more to be regretted, as the editor is clearly sensitive to the issues involved. Indeed, for this reason, Mr Williams has gone outside the ranks of the technological *cognoscenti*, and has invited several chapters of contextual discussion — including an historical overview from T.K. Derry, and chapters on innovation by David Sawers; on the economics of technology by Frank Bradbury; on management by Glenn Porter; on trades unions by Harold Pollins; on education by David Layton; on government by Alexander King; and on the chemical industry by Lutz Haber. These contributions are of varying quality (Haber's and Layton's in particular deserve a wider readership), but whatever their individual merits, they do not together weave a comprehensive fabric. Written, one supposes, in isolation from each other, they largely fail to reveal the depths of controversy reigning within these areas of discourse. The explanations offered are, in many ways, too consensual, too safe, and altogether too undemanding. When they jar, then, like a clock striking thirteen, they cast doubt on all else. For example, David Sawers concludes provokingly that "the nature of . . . technology appears to influence the nature of the industrial structure more than the structure influences the technology". Surely, the two volumes of evidence which follow could, if skilfully used, demonstrate, disprove, or at least illuminate this assertion. At the very least, the economic and social analysis of new technologies cannot be merely relegated to separate chapters. At a more philosophical level, far more could be said about the relationship between technology and cultural expectations. For example, it could be argued (with important qualifications) that a presumed alliance between liberal values and technological growth was widely considered axiomatic in the 19th century; but Derry's passing reference to this fails to demonstrate why this should have been so, or what relevance

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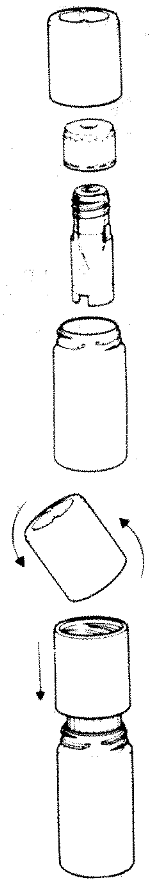

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this belief in "technologism" had to subsequent political and social development. Here and elsewhere historical judgements will not escape historical criticism. And in the meantime, many important relationships — between technology and empire, between political and "technological dependence", between civilian and military technology, and between the "technostructures" of different economic systems — are simply not adequately treated.

Browsing through these excellently produced volumes, the reader may, at length, ask a third question. Social history, Trevelyan once remarked, is history with politics left out. The history of technology is, traditionally, history with the people left out. Beyond inventors, corporations and eponymous effects, this *History of Technology*, like its predecessors, largely fails to situate technological developments at the level of human beings caught up and affected by them. This separation between the word and the thing has until recently rendered much of the history of technology an arid discipline, often bereft of human reality. It has been by all accounts a *hasard du métier*. But the time has come to catch up, at least, with recent historiography. In these volumes, revealingly, the excellent photographs wordlessly describe much of the reality that the text leaves unstated. Even to the casual reader, pictures of working conditions in the paint industry (Fig. 23.3) rest uneasily next to discussions of chemical development. And one supposes that the plate (Figs.21.15 and 21.16) which offers an ironic (and unintended?) juxtaposition of *men* in lab coats in Levenstein's Manchester works (about 1912) alongside *women* in overalls cleaning chemical casks (about 1943) will not pass unnoticed by students of technology and the labour process. Nor, for that matter, will feminist historians leave unchallenged the chapter on domestic appliances, which manages to describe a host of new inventions, and mentions more than once the "servant class", without once referring to the commonplace contradictions of "liberating technology" in modern society.

Methodologically, it can be argued that the omnibus, encylopaedic approach to technology represented in these volumes, has had its day. What of the future? In the last chapter, written by the editor, there is a beginning. Mr Williams, as a good historian, looks forward, and in his summary he sees the chief lesson of the past and the problem of the future as basically one of choice — one of selection — between different technologies. Other historians, economists and even technologists today would argue that the real problem, no less pressing in the Third World than in the West, is not merely one of choice between different technologies, but of choice between different styles of life. Mr Williams sees clearly the difficulty of first defining an acceptable goal, then creating the technology necessary to achieve it. But to do something like this, after all, is surely part of the technologist's credo. And in failing to set and observe clear social priorities, technology in the West has been allowed to become less a means and more an end in itself.

Historians now widely recognise that it is the fate of technology not to have an ideology, but to be one. As Langdon Winner has recently observed, the fear that technologies and the factors governing them have become 'autonomous' outside the rational control of governments, or engineers, is part of the Western condition. There is no reason, on current calculations, why this fear should not also one day confound large parts of the Third World. Since *Silent Spring*, the environment, and since the oil crisis, energy, have become the two principal epicentres of disquiet with technology. But the historical and philosophical issues raised in *A History of Technology* are not confined to these matters alone. That these last volumes are edited by Trevor Williams alone, no longer aided by a large industrial subsidy, itself speaks volumes for the change in our circumstances.

When they turn back to his *History* for technical answers, readers of these volumes will be left asking important questions. One hopes they will find the editor sympathetic. Is it sufficient, today, for example, to deplore alleged 'constraints' on 'progress', when one is forcibly reminded almost every day of the traumatic story of nuclear power from Los Alamos to Harrisburg? If not in the 1950s, then from the 1970s some conception of 'open' technology will require a place alongside participatory access to 'open government'. In the process it is not only the technologist and the politician, as Mr Williams suggests in an adventitious pun, whose resources will be taxed in the search for solutions. If the future lies in the discovery of alternatives, solutions can lie only in ourselves.

R. M. MacLeod

R.M. MacLeod is Chairman of the Department of Science Education at the University of London Institute of Education, London, UK.

Hot-atom reactions

Chemical Effects of Nuclear Transformation in Inorganic Systems. Edited by G. Harbottle and A. G. Maddock. Pp.540. (North-Holland: Amsterdam, London and New York, 1979.) $87.75; Dfl. 180.

ATOMS undergoing almost any nuclear process are characterised by excitation energies usually far in excess of the energies associated with chemical processes. The excitation may be in the form of kinetic or electronic energy (including ionisation), the amounts depending on the nature of the nuclear process occurring. If the process gives a radioactive atom, the atom is conveniently 'tagged' at birth and its subsequent fate readily followed.

The chemical effects of the nuclear process are firstly to cause rupture of some or all of the bonds in which the atom was involved and secondly to allow the atom to undergo novel types of reaction not normally observable in conventional chemical systems. These are normally called 'hot atom' reactions, as the excitations involved correspond to 'temperature' of the order of 10^5K or higher.

The present volume is a comprehensive survey of these reactions occuring in inorganic systems, up to the mid-1970s. It has 27 chapters contributed by 28 authors and lists some 1900 references in total. The limitation to inorganic systems may seem somewhat arbitrary, but in fact the coverage of the whole field is good and few if any topics of fundamental importance are omitted.

After a short historical introduction the first few chapters deal with the primary processes following nuclear activation and reactions in the gas phase. Gas phase studies have contributed significantly to our understanding of high energy reactions through the work of Wolfgang, Rowland and others. However, as most inorganic compounds are not gaseous , the vast majority of the work covered here deals with the condensed phases, especially solids. Much of the work on solids has been stimulated by the phenomemon of 'annealing' in which the activated atom, having undergone bond rupture during nuclear activation, returns to the form of the parent compound as a result of a variety of treatments, such as heating or gamma irradiation.

The division of the chapters is made on broadly chemical criteria, so that each group of compounds is treated separately. This makes sense because by far the most popular method of nuclear activation is the (n, γ) reaction and there are separate chapters dealing with other nuclear processes such as isomeric transition, beta decay, fission fragments, and so on.

A major experimental problem in studies on solids is the need to dissolve the solid in order to analyse products. The possibility of reactions during or after dissolution makes it difficult to be sure of the precise nature of the species present in the solid. Numerous attempts to overcome this difficulty have been made and some of these are reported here. One obvious approach is direct studies on the solid itself, but this is difficult because of the exceedingly low concentration of radioactive atoms usually present. Mössbauer and related techniques have yielded some information in this area, but by no means solve the problem.

In summary, this volume provides a comprehensive study of the state of the art in this subject. It accurately reflects the uncertainties as well as the progress made. The standard of treatment is somewhat variable, as might be expected with such a large number of contributors, but the editors have done an excellent job in avoiding overlaps and repetition. The price will probably put the book beyond the reach of even the most enthusiastic individual, but it should certainly find its place in libraries. **V. J. Robinson**

V. J. Robinson is Lecturer in Radiochemistry at the University of Manchester, UK.

Energetic Mancunian

James Prescott Joule and the Concept of Energy. By H.J. Steffens. Pp. 172. (Neale Watson: New York; Dawson: Folkstone, UK, 1979.) $20; £10.

NEARLY a century ago a reviewer in the *Philosophical Magazine* wrote:

> "Not Copernicus and Galielei, when they abolished the Ptolemaic system; not Newton, when he annihilated the Cartesian vortices; not Young and Fresnel, when they exploded the Corpuscular Theory; not Faraday and Maxwell, in their splendid victory over *Actio in distans* — more thoroughly shattered a malignant and dangerous heresy, than did Joule when he overthrew the baleful giant FORCE, and firmly established, by lawful means, the beneficient rule of the rightful monarch, ENERGY! Then, and not till then, were the marvellous achievements of Sadi Carnot rendered fully available; and Science silently underwent a revolution more swift and more tremendous than ever befell a nation".

None of this Victorian enthusiasm for the Manchester brewer's son is conveyed by Henry Steffens, nor does he attempt to explain the significance of the change from "force" to "energy" in physics. He has compiled instead a sober survey of Joule's experiments and theories from 1837 to 1852, together with a discussion of closely related contributions of J.R. Mayer, William Thomson, W.J.M. Rankine, R. Clausius and H. von Helmholtz. Steffens elucidates the path from early experiments on the "electromagnetic engine" to determinations of the mechanical equivalent of heat and formulations of the first law of thermodynamics. Although it provides a clear and accurate account of one aspect of the history of energy conservation, the book suffers from its limited scope. We learn little about Joule's personality or his influence beyond the small circle of scientists mentioned above, and scarcely anything about the concept of energy apart from Joule's rather narrow interpretation of it.

Steffens' most interesting chapter is the one dealing with Joule's rough translation, in his notebook, of Mayer's 1842 paper; he argues that Joule knew of Mayer's work before 1844 and was thereby led to perform the experiments on rarefaction and condensation of air that yielded his third value for the mechanical equivalent of heat. But aside from this, Steffens makes very little use of documents, even those already published (for example, by A.L. Hatton and L. Rosenfeld in 1956, and by J.T. Lloyd in 1970).

This book is almost identical to the author's 1968 dissertation, with the addition of four pages on the paddle-wheel experiment and the deletion of some material on Mary Somerville and W.R. Grove in chapter 6. Much has been written on nineteenth-century physical science during the past decade and as a result historians of science have not only a different view of the development of energy concepts but an enhanced interest in philosophical and sociological questions. Steffens mentions some of the relevant publications in his notes but has made few substantive changes in his text. Perhaps most serious is his failure to take account of John Forrester's important article on the chemical background of Joule's work (*Studies in History and Philosophy of Science*, 1975). Ten of the 22 items in Forrester's "Joule bibliography" are missing from Steffens' bibliography (users of the latter should be warned of a mixup of lines of type on pages 165-66).

It is necessary to criticise two features which this book shares with an increasing number of scholarly monographs. First, if notes must (because of the deterioration of printing technology) be placed at the end of the book instead of the foot of the page, they should be keyed to the text pages so one does not have to find out what chapter one is reading in order to locate its notes. Second, the index should cover the notes as well as the text; in this case it doesn't even cover the text adequately (for example, Aepinus has no entry).

Stephen G. Brush

Stephen G. Brush teaches history of science at the University of Maryland, College Park.

Liquid crystalline materials

Liquid Crystals and Biological Structures. By G.H. Brown and J.J. Wolken. Pp. 187. (Academic: New York, San Francisco and London, 1979.) $19; £12.35.

IT is surprising that many distinguished scientists, including Bernal in 1933 and Needham in 1950, should have pointed out so early the importance of the liquid crystalline state for biological systems. Yet it was only in the early 1960s that a major attempt was made to examine experimentally the liquid crystalline properties of biological molecules. Nowadays when so many scientists have contributed to the study of phospholipid-water systems to provide insight into biomembrane structure and function, it is interesting to remember that a great deal of the basic fundamental work — for example, phase transitions, phase diagrams, fluidity, bile salts and cholesterol effects — was carried out as recently as the 1960s. When we further note that myelin figures were first observed by Virchow in 1854 it is interesting to consider the socio-economic technological factors which held up the study for so long of these important liquid crystalline lipid-water systems.

Brown and Wolken remind us of this in the present book. They examine the basic properties of liquid crystalline materials and include a discussion of the various classifications, including nematic, cholesteric and smectic liquid crystals. Thermotropic and lyotropic liquid crystal and the neat soap phase important to the detergent industry is shown. The optical properties of liquid crystals are also adequately but briefly described. These chapters I found neat, and useful. However, the later chapters dealing with the relationship of these fundamental properties of matter to the biological structures are less satisfactory.

The authors include chapters on the cell, photoreceptor structures (that is, chloro-photoreceptor structures, that is, chloroplasts and the eye, fibrous protein structures and effectors, and biomembranes. They also include applications to medicine, such as temperature scanning and diseases. I found these biological chapters a little too superficial for my taste. I would have preferred some better examples of how important an understanding of liquid crystals has been for their biological implications — for example, the way in which thermotropic phase transitions which occur in pure phospholipid-water systems are clearly observed in some natural biomembranes. The use of liposomes as potential pharmacological capsules and the formation of gall-stones are also given only passing reference, and yet these are interesting, useful and important applications of the study of liquid crystalline substances to biology and medicine.

As an overview providing a stimulus for a student or young research worker the book may be valuable. It is clearly written with good diagrams and printing. I observed only a few obvious typographical errors.

Dennis Chapman

Dennis Chapman is Professor of Biophysical Chemistry at the Royal Free Hospital School of Medicine, University of London, UK.

obituary

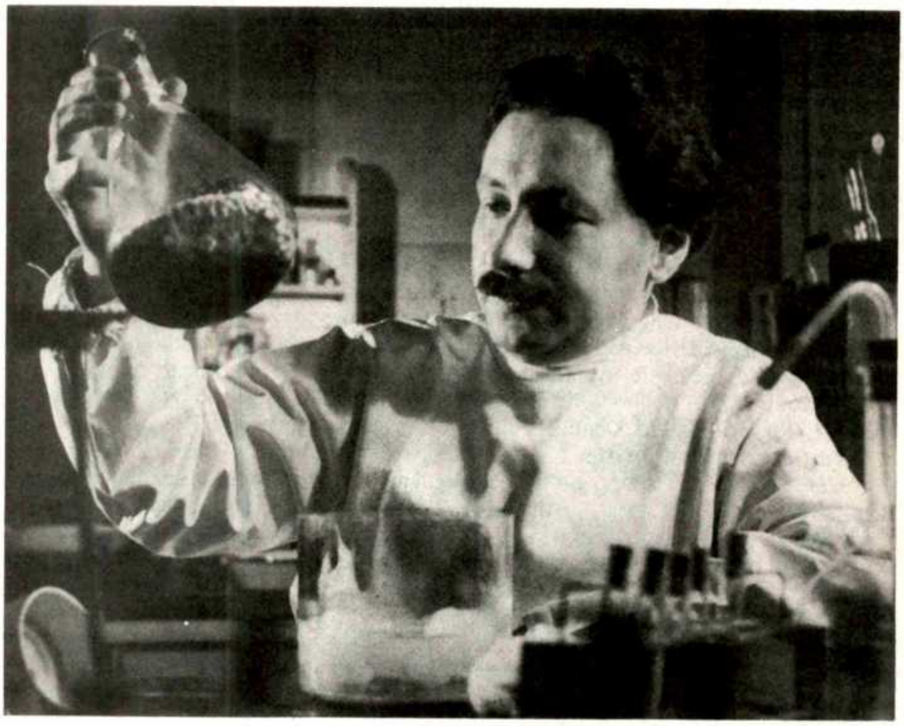
Sir Ernst Chain, 1906-1979

Sir Ernst Boris Chain, who received the Nobel Prize for Physiology and Medicine with Fleming and Florey in 1945, for the discovery and isolation of penicillin, died on 12 August 1979 while on holiday at his country home in Ireland.

He was born in Berlin of a Russian emigré father and a German mother. On 30 January 1933, Chain left his native Berlin to escape from the mounting evils of Nazi Germany. He arrived in London with little but hope and a determination to follow one or other of his great passions — music or science. After a short interlude at University College London, Chain had the good fortune to be recommended by J.B.S. Haldane to Sir Frederick Gowland Hopkins, then Head of the Sir William Dunn School of Biochemistry at Cambridge. Chain was overjoyed with this unexpected opportunity which he seized eagerly: the die was cast on the side of science rather than music.

The topic which engaged Chain's interest was the biochemical basis of the neurotoxic effects of snake venoms. At Cambridge, Chain found that some of the most potent venoms were able to inhibit alcoholic fermentation and glycolysis when added in very small amounts to cell-free extracts. Further studies showed that this was due to destruction of an essential coenzyme by simple hydrolysis. This approach of biological observation followed by biochemical explanation was symptomatic of Chain's scientific philosophy which he followed consistently through 45 years of amazingly diverse achievements.

After two years of working with 'Hoppy', Chain was approached by Professor Howard Florey with an invitation to join Florey's staff at the Sir William Dunn School of Pathology at Oxford. Florey had no biochemical training but he did have an awareness of the importance of biochemistry in the understanding of all biological sciences and in particular of his own field of experimental pathology. Florey drew Chain's attention to an observation by Fleming in 1924 concerning the lytic properties of tears, nasal secretions, and egg white on bacteria. Such a problem — to study the biochemical mode of action of a powerful bacteriolytic agent — was typical of the Chain approach. He was able to show that the lytic properties were due to lysozyme which acted upon a bacterial polysaccharide.



Chain in Oxford, c. 1940

While preparing this work for publication in 1937, and arising from discussions with Florey, Chain stumbled upon the concept of growth inhibition by bacteria, fungi and yeasts acting on one another, including Fleming's neglected original report on the bacteria-inhibiting properties of *Penicillium notatum*. Upon starting their systematic investigation of the antibacterial substances produced by micro-organisms, Florey and Chain were immediately embarassed by a severe shortage of funds compounded by the refusal of the Medical Research Council to fund the work. One day in the autumn of 1937, Chain received a message from Florey stating that in view of the fact that the department had an overdraft of £500 no further equipment of any kind was to be ordered — not even a glass rod!

This experience had a profound effect on the young, enthusiastic Chain who was impatiently waiting to unleash his energies on the new problem of explaining in chemical terms how bacterial growth was inhibited. The unsuccessful approach to the Medical Research Council reinforced his suspicions about university and government financial support. Since that day, he never allowed himself to become wholly dependent for research funds on political largesse, however respectable its facade. Undeterred by this initial setback, Chain discussed with Florey a grant application to the Rockefeller Foundation to cover the costs of the proposed programme for a number of years, so that financial stability could be ensured. To Chain's great delight, the Rockefeller Foundation were sympathetic towards biochemical research and granted him $5,000 for five years.

The penicillin story is well known as it culminated in a Nobel Prize for Chain, Fleming and Florey in 1945. Chain was not the type to rest on his laurels. He was incensed by the refusal of the MRC and Oxford University to consider any form of patent protection for penicillin which was subsequently developed commercially in America. Chain also became increasingly frustrated by the problems of obtaining adequate facilities for the fermentation of micro-organisms on a semi-industrial scale. The methods used initially to provide impure penicillin for laboratory studies and even clinical trials were crude. Chain was very outspoken about the amateurish approach in Britain which he contrasted with the facilities available in the USA. He campaigned vigorously at all levels, for fermentation pilot-plant facilities, but he was met by the implacable inertia of the Establishment who made it very clear that such facilities were a matter for industry and had no place in an academic ivory tower. Chain poured scorn on the view that the size of the reaction vessels should determine whether the biochemical problem to be investigated was 'pure' or 'applied'. In the true British spirit of compromise, Chain was finally offered a single fermenter of moderate dimensions. Chain's reply was to ask if an organic chemist would be prepared to limit himself to the use of one Erlenmeyer flask!

More in sorrow than in anger, Chain accepted in 1948 an invitation to organise a department of biochemistry at the Italian State Institute of Health in Rome. The temptation was made irresistable by the promise that in Rome, an International Centre for Chemical Microbiology containing the controversial fermentation pilot plant with extraction and purification facilities would be made available. The magnificent facilities and the collaborations with Ballio, Dentice, Falini, Marotta (the Director of the Institute), Pocchiari and his colleagues, produced a rich harvest of publications in the new science of chemical microbiology and on the biochemical mechanisms of hormonal control. A constant stream of scientific visitors passed through Chain's laboratories in Rome, so that at any one time at least half-a-dozen different nationalities were represented.

At the start of his Italian era Chain married Anne Beloff in 1948. His wife, who had trained under Sir Rudolf Peters and Baird Hastings, had an active team of collaborators in Rome investigating the mode of action of insulin. During this period the Chains were delighted by the birth of their three children — Benjamin, the eldest son, and then twins, Judith and Daniel.

In 1940 Abraham and Chain had identified the enzyme penicillinase, which inactivates penicillin and which is produced, with great foresight, by numerous bacteria. Chain felt that the affinity of penicillin for this bacterial enzyme could be altered if the penicillin molecule could be chemically modified. Enormous effort had already been expended in the pharmaceutical industry in the search for chemical precursors acceptable to the *Penicillium* mould in the hope that novel penicillins with useful properties would be biosynthesised. Of these, phenoxymethylpenicillin (penicillin V) had turned out to be acid stable and useful clinically because of its absorption after oral administration. Apart from this limited success, some disillusionment had set in regarding the prospects of obtaining novel penicillins, and many laboratories had turned their attentions to more promising areas of antibiotic research. Chain's idea was to improve upon nature by persuading the mould to grow *p*-aminobenzyl-penicillin and then using the chemically reactive amino group for further man-made chemical modifications.

Chain was approached in 1954 by the then Chairman of the Beecham Group, Henry Lazell, on the advice of the late Sir Charles Dodds, with a view to advising Beechams on entering the antibiotics field. Chain's enthusiasm and drive so impressed Lazell that Beechams soon found themselves with a fermentation pilot plant looking remarkably like the one in Rome, and a research programme aimed at modifying *p*-aminobenzyl-pencillin. A small team of scientists was sent to Rome by Beechams while the fermentation plant was being erected. During their stay in Rome in 1956, an interesting difference in the results of two assays of the crude microbial culture fluids was noticed. The chemical assay based on the behaviour of the fused thiazolidine-β-lactam ring consistently gave higher results than the bioassay which required the full penicillin molecule for biological activity. An explanation of this finding followed when the team returned to Beecham Research Laboratories in 1957 when they demonstrated that the discrepancy was due to the presence of the penicillin nucleus (6-aminopenicillanic acid) which was subsequently isolated and characterised, (*Nature*, **183**, 257, 1959). The gateway was now open to an enormous variety of chemically modified derivatives beyond all the original expectations based on the limited synthetic variations possible with *p*-aminobenzyl-penicillin.

The focal point of antibiotic research had moved back to England, and this coincided with a worsening of the political situation in Rome. In the course of a visit to Rome in 1958, Professor P.M.S. Blackett discussed with Chain the possibility of setting up at Imperial College, London, a comprehensively equipped biochemistry department modelled along the lines of the facilities available at the Institute. A further visit by the then Rector, Sir Patrick Linstead, led to a tentative proposal that Chain should return to Enland to re-establish biochemistry at Imperial College where the chair had been vacant since Chibnall left for Cambridge in 1943.

The cost of all the fermentation equipment, instrumentation, electrical and mechanical workshops, required to support and service the complex chemical microbiological research and metabolic biochemical investigations to be undertaken by Chain, was formidable and unheard of in the academic world outside the realms of nuclear physics. The scheme was shelved. However, Chain accepted the challenge of an apparently insoluble difficulty and campaigned vigorously to find independent funds for this imaginative project which complemented the already very strong chemical and chemical engineering interests of Imperial College. As a result of Chain's efforts and Linstead's encouragement, the Wolfson Foundation provided £350,000 to establish the new Wolfson Laboratories, the Science Research Council granted £110,000 in support of fermentation research, and the Fleming Memorial Fund granted Chain £50,000 for basic medical research. Chain had not forgotten the lesson of the forbidden glass rod at Oxford!

Chain returned to take up his new appointment in October 1964 and the new laboratories were completed in the summer of 1965. The Medical Research Council had buried the hatchet by establishing a Metabolic Reactions Research Unit under Chain. Dr Anne Beloff-Chain also continued her studies on insulin and the control of carbohydrate metabolism in the new department as Reader in Metabolic Biochemistry. The financial independence which Chain secured from the very conception of the new department did nothing to endear him to the administrative establishment which found him unimpressed by exhortations to economise in lean years. Minor officials in the Science Research Council and University Grants Committee secretariat found their cautious criticisms of Chain's eminently unreasonable demands quoted, or even possibly misquoted, by Chain in direct conversations with the Treasury or the Prime Minister (according to availability). It would be wrong however to give the impression that Chain was a mere irascible crusader against bureaucracy. He had an affectionate regard for his circle of friends with whom he loved to share musical evenings in which their active participation was expected. He was devoted to his family and all visitors, however distinguished, were put aside, albeit temporarily, whilst Chain greeted his sons and daughter when they called in to his office on their way home from school.

The premature death of Sir Patrick Linstead was a sad personal loss to Chain and a watershed in the fortunes of the department. The sympathetic understanding which Chain had enjoyed with Linstead was irreplaceable and the special relationship of the department, because of its high level of collaboration with industry and support by charitable institutions, became almost an embarassment in inter-college negotiations for funds.

Chain's knighthood came as something of a surprise in 1969 — not, of course, because of any doubts about his quite exceptional personal achievements over 35 years, but because his deliberate policy of rocking Establishment boats had made a deep impression in what are termed 'influential quarters'. It says something for the British political system that his scientific genius finally triumphed over his political reputation, even though 20 years earlier his election to Fellowship of the Royal Society had been delayed for several years.

After his retirement from the chair at Imperial College in 1973, he became Emeritus Professor. He continued to travel widely, aided by his ability to lecture in five languages. Honours and decorations came from many Universities and Governments including France, Italy and Japan, and Chain continued his long, active association with the Weitzman Institute as a Governor.

An abiding memory for his family and friends must be the marvellous occasion in 1976 when Sir Ernst was guest of honour at a dinner held at the Middle Temple Hall to mark his 70th birthday. A crowded scientific programme had been given at the Royal Society over the preceding two days by his closest collaborators. This programme reflected Sir Ernst's research contributions in food technology and non-specific immunity as well as microbial and physiological biochemistry. Fortuitously news came through of his eldest son's first class honours in zoology at Cambridge. After dinner and the speeches (Sir Ernst almost lost for words for once), the entertainment for the distinguished international audience was opened by Sir Ernst and Benjamin Chain playing piano duets with great panache and empathy — a fitting reminder that the gain of science and medicine from the work and life of Ernst Chain was a loss to the concert hall and the world of music.

K. R. L. Mansford

Sir Harry Champion

SIR HARRY CHAMPION, CIE, Emeritus Professor of Forestry in the University of Oxford, died on 19 June 1979. He was born in 1891 and was educated at the Royal Grammar School, Guildford. He was at Kings College, London University, for a year before going up, as a Commoner, to New College, Oxford in 1909. The subsequent award of a college scholarship was the first of his many academic achievements at Oxford. In 1912 he took a first in chemistry, in 1913 a first in botany and in 1914, under William Schlich, a Diploma in Forestry with distinction.

In 1912 he was accepted as a probationer in the Indian Forest Service and after spending a year in the United States on a Carnegie Travelling Scholarship to study entomology he went to India in October 1915.

Champion was appointed Assistant Conservator of Forests in the United Provinces, where P.H. Clutterbuck, later Inspector General of Forests, was Chief Conservator. At an early stage in his career he demonstrated clearly his versatility. In 1916 he was Acting Forest Entomologist at the Forest Research Institute, Dehra Dun, and in 1921 Silviculturist for the United Provinces. As a District Officer he compiled a check list of the fauna of the West Almora Division and also effectively modified the condensing equipment on plant used to distill tar from Chir pine. For the latter he was commended by the Governor of the Province. He was mentioned in other reports for extracting the maximum amount of wood from a very difficult location. At this time he also showed that twisted fibre in Chir pine, a very damaging defect of this species, was a heritable character and not an effect of site conditions.

For the academic year 1924/25, while on leave pending retirement, he held the post of Silviculturist under Troup at the Imperial Forestry Institute, Oxford. In 1925 he married Chrystal Parsons, the grand-daughter of an Indian Army officer and decided to return to India — a momentous decision.

From 1925 until 1936 Champion was seconded to the Department of Education, Health and Lands of the Government of India and appointed Silviculturist at the Forest Research Institute, Dehra Dun. As Central Silviculturist he carried out research in a great variety of silvicultural projects, including natural forest regeneration and the establishment of plantations, as well as engaging in the preparation of yield tables and entomological problems.

He travelled extensively in India, and visited Burma and the Andaman Islands. In 1934/35 he was loaned to the Government of Ceylon to advise on forestry. From his experimental work and travelling he acquired an unparalleled knowledge of Indian forestry. During this period he also toured in the United States, Canada and the Far East. He visited most of the European forest research establishments and established himself as a forester of international repute.

In 1937 Champion returned to the United Provinces where he held the posts of Divisional Forest Officer, Conservator of Forests and Conservator of Working Plans acquiring experience of forest administration at senior level. He seemed destined to go to the top in the Indian Forestry Services, but in 1940 again changed the course of his career by accepting the Chair of Forestry at Oxford and the directorship of the Imperial Forestry Institute, as successor to Troup.

Champion's first notable achievement at Oxford was the establishment, in 1944, of an Honours School of Forestry. As Professor of Forestry he was *ex officio* Consultant Director to the Commonwealth Forestry Bureau and provided for the first Director, J.W.B. Sisam, and later Henry Ford Robertson, encouragement, advice and support to establish that most useful and efficient organisation. He also strengthened the scientific basis of teaching and research in forestry by the appointment of biological scientists. Between 1940 and 1959 he travelled extensively with forestry classes on the continent, on advisory missions and lecture tours to most of the British colonies, and to attend international conferences. His experience in forestry was then world wide and he continued to make best use of it by writing and advising.

After his retirement in 1959 he was appointed Emeritus Professor in 1960 and until 1967 undertook further advisory missions and consulting assignments. In 1962/63 he was engaged by the Government of India to revise the silvicultural textbooks he had written when at Dehra Dun and had a similar assignment for the Government of Pakistan in 1963/64.

Champion published work, mainly in the Indian Forest Records, on every aspect of forestry, including research methods, statistics, working plans and ecology as well as silviculture. His interest in research methods is apparent from his *Silvicultural Research Manual for India, Vol.I: General*, and *Vol.II* (with I.D. Mahendru): *Statistical Code*, 1931. The results of his experimental work and his general observations were incorporated in a *Manual of Indian Silviculture* (with Sir Gerald Trevor) which has now been revised twice (in 1968 with S.K. Seth). In 1965, together with C.M. Khattak and S.K. Seth he prepared a similar manual for Pakistan. Of necessity, most of his time was devoted to matters of immediate and practical importance, but he was very concerned about the scientific basis of forestry and his *Forest Types of India and Burma* (1931, revised with S.K. Seth in 1968) is a major contribution to forest ecology.

Champion's work in India was acknowledged in 1941 by the award of Companion of the Order of the Indian Empire and his scientific achievements by an Oxford DSc in 1950. For his services to forestry in general he was made a Knight Batchelor in 1956. He was chairman of the Commonwealth Forestry Association from 1959 to 1961, president of the Society of Foresters of Great Britain in 1958 and 1959 and an honary member of many national forestry societies. In 1969 he was given the Fernow Medal, awarded jointly by the American Forestry Association and the German Forestry Society.

Twenty classes of Oxford undergraduates and hundreds of foresters from every part of the world have been entertained in unique style by the Champions at Windrush. Some of us that were members of his earlier classes have further reason to be particularly grateful to them for many other kindnesses, among them dispensation to bring, what Chrystal Champion called our "probationary forest wives", on field tours.

Finally it is my privilege to put on record the immense contribution made by Chrystal Champion to the life and achievements of Harry Champion. She was keenly interested in his work, shared his fondness for travelling and perhaps persuaded him to return to India. It was a perfect match and good for forestry.

J.F. Hughes

Recent scientific and technical books

Mathematics

BYRNES, Christopher I. (ed.). Partial Differential Equations and Geometry. (Proceedings of the Park City Conference.) (Lecture Notes in PUre and Applied mathematics, Vol. 48.) Pp.x + 323. ISBN-0-8247-6775-6. (New York and Basel: Marcel Dekker, Inc., 1979.) Sw.fr.78.

CIGLER, Johann, LOSERT, Viktor, and MICHOR, Peter. Banach Modules and Functors on categories of Banach Spaces. (Lecture Notes in PUre and Applied Mathematics, Vol.46.) Pp.xv + 282. ISBN-0-8247-6867-1. (New York and Basel: Marcel Dekker, Inc., 1979.) Sw. francs 66.

GERAMITA, Anthony V., and SEBERRY, Jennifer. Orthogonal Designs: Quadratic Forms and Hadamard Matrices. (Lecture Notes in Pure and Applied Mathematics, Vol.45.) Pp.x + 460. ISBN-0-8247-6774-8. (New York and Basel: Marcel Dekker, Inc., 1979.) SFr. 78.

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LOJDA, Z., GOSSRAU, R., and SCHIEBLER, T.H. Enzyme Histochemistry: a Laboratory Manual. Pp.vii + 339. ISBN-3-540-09269-2. (Berlin and New York: Springer, 1979.). DM 72; $39.60.

McFALLS, Jr., Joseph A. Psychopathology and Subfecundity. (Studies in Population.) Pp.xix + 264. ISBN-0-12-484650-5. (New York and London: Academic Press, 1979.) $18.

MEIER-RUGE, W. (ed.). CNS Aging and Its Neuropharmacology: Experimental and Clinical Aspects. (Interdisciplinary Topics in Gerontology, Vol.15.) Pp.viii + 220. ISBN-3-8055-2980-5. (Basel, London and New York: S. Karger, 1979.) SFr.88; DM 105; $52.75.

MITCHELL, John, and ROOK, Arthur. Botanical Dermatology: Plants and Plant Products Injurious to The Skin. Pp.xiii + 787. ISBN-0-88978-047-1. (Vancouver: Greenglass, Ltd.; London: Henry Kimpton Publishers, 1979.) £30.

MOUNT, Laurence E. Adaptation to Thermal Environment: Man and his Productive Animals. (A Series of Student Texts in Contemporary Biology.) Pp.xiii + 333. ISBN-0-7131-2740-6. (London: Edward Arnold (Publishers), Ltd., 1979.) Boards £16; Paper £7.50.

NAHAS, Gabriel G. Keep Off the Grass: a Scientific Enquiry into the Biological Effects of Marijuana. Pp.xviii + 259. ISBN-0-08-023780-0. (Oxford and New York: Pergamon Press, 1979.) Hard £7.50; Flexi £3.95.

OSTRANDER, Lee E. (ed.). Proceedings of the Seventh New England (Northeast) Bioengineering Conference, March 22-23, 1979, Rensselaer Polytechnic Institute, Troy, New York. Pp.xxiii + 530. ISBN-0-08-024634-6. (Oxford and New York: Pergamon Press, 1979.) £27.50.

PANAYI, G.S., and JOHNSON, P.M. (ed.). Immunopathogenesis of Rheumatoid Arthritis. (Proceedings of the International Symposium held at Guy's Hospital, London in November 1978.) Pp.163. ISBN-0-906544-02-5. (Chertsey, Surrey: Reedbooks, Ltd., Fox Lane North, 1979.) £19.

PRASAD, Ananda S. Trace Elements and Iron in Human Metabolism. Pp.xv + 392. ISBN-0-471-99761-7. (Chichester and New York: John Wiley and Sons, 1978.)

PRESTON, Jr., Kendall, TAYLOR, Kenneth J.W. JOHNSON, Steven A., and AYERS, William R. (ed.). Medical Imaging Techniques: a Comparison. Pp.xxiii + 372. ISBN-0-306-40161-4. (New York and London: Plenum Press, 1979.) $37.50.

REITZ, ROSETTA. Menopause: a Positive Approach. Pp.xi + 276. ISBN-0-85527-964-8. (Hassocks, Sussex: The Harvester Press, Ltd., 1979.) £4.95.

RUDOLPH, Ross, FISHER, Jack C., and NINNEMANN, John L. Skin Grafting. Pp.xi + 205. ISBN-0-316-76109-5. (Boston, Mass: Little, Brown and Company, 1979.) $16.50.

SCANU, Angelo (ed.). In association with WUSSLER, Robert W., and GETZ, Godfrey S. The Biochemistry of Atherosclerosis. (The Biochemistry of Disease: a Molecular Approach to Cell Pathology, Vol.7.) Pp.xx + 548. ISBN-0-8247-6767-5. (New York and Basel: Marcel Dekker, Inc., 1979.) Sw.Fr.112.

SCARPELLI, Emile M., and COSMI, Ermelando V. (ed.). Reviews in Perinatal Medicine, Vol.3. Pp.x + 483. ISBN-0-89004-249-7. (New York: Raven Press, 1979.) $36.

SCAVIA, Donald, and ROBERTSON, Andrew (ed.). Perspectives on Lake Ecosystem Modeling. Pp.xiv + 326. ISBN-0-250-40248-3. (Ann Arbor, Michigan: Ann Arbor Science Publishers, Inc.; 1979. Distributed in UK by John Wiley and Sons, Chichester.) £14.85.

SCHNEIDER, Ferdinand (ed.). Sugar Analysis. (Official and Tentative Methods Recommended by the International Commission for Uniform Methods of Sugar Analysis, ICUMSA.) Pp.xiv + 265. ISBN-0-905003-01-2. (Peterborough: ICUMSA, 1979). £12.

SIMMONDS, N.W. Principles of Crop Improvement. Pp.xiv + 408. ISBN-0-582-44630-9. (London and New York: Longman, 1979.) £7.95.

SNEEP, J., HENDRIKSEN, A.J.T. (ed.). HOLBEK, O. (coed). Plant Breeding Perspectives. (Centennial Publication of Koninklijk Kweekbedrijf en Zaadhandel, D.J. van der Have, 1897-1979). Pp.xxv + 435. ISBN-90-220-0697-2. (Wageningen: Centre for Agricultural Publishing and Documentation, 1979.) Dfl.120.

SOME MONOMERS, PLASTICS AND SYNTHETIC ELASTOMERS, AND ACROLEIN. (IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol.19.) Pp.513. ISBN-92-832-1219-3. (Lyon: International Agency for Research on Cancer, 1979. Distributed by WHO, Geneva; HMSO, London.) Sw.fr.60.

VAUGHAN, J.G. (ed.). Food Microscopy. (Food Science and Technology: a Series of Monographs.) Pp.xvi + 651. ISBN-0-12-715350-0. (London and New York: Academic Press, 1979.) £29.60; $61.25.

VISSER, H.K.A. (ed.). Nutrition and Metabolism of the Fetus and Infant. (Fifth Nutricia Symposium, Rotterdam, 11-13 October 1979.) Pp.xvi + 419. ISBN-90-247-2202-0. (The Hague, Boston, Mass. and London: Martinus Nijhoff Publishers, 1979.) Guilders 110.

WALSH, Christopher. Enzymatic Reaction Mechanisms. Pp.xv + 978. ISBN-0-7167-0070-0. (San Francisco and Reading: W.H. Freeman and Company, 1979.) £18.30.

WALTER, Hartmut. Eleanor's Falcoln: Adaptations to Prey and Habitat in a Social Raptor. (Wildlife Behaviour and Ecology.) Pp.xiii + 410. ISBN-0-226-87229-7. (Chicago and London: The University of Chicago Press, 1979.) $43.75; £24.50.

WILLIAMS C.N. The Agronomy of the Major Tropical Crops. Ppxix + 228. ISBN-0-19-580296-9 (Oxford: Oxford University Press, 1979.) £8.50.

WILSON, James A. Principles of Animal Physiology. Second edition. Pp.xviii + 891. ISBN-0-02-428360-6. (New York: Macmillan Publishing Co., Inc.; London: Collier Macmillan Publishers, 1979.) $30.20; £16.45.

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APPOINTMENTS VACANT

THE MIDDLESEX HOSPITAL MEDICAL SCHOOL

SCHOOL OF PATHOLOGY

MEDICAL LABORATORY SCIENTIFIC OFFICER HISTOLOGY

A skilled, experienced histology MLSO required as soon as possible to join a research team studying carcinogenesis in vitro in organ cultures. Preference will be given to someone able to section plastic embedded- as well as wax embedded-material for high resolution histology. Candidates should be Associates of the IST, IMLT or equivalent. The appointment is for one year initially, renewable for a further two. Salary in the range £4,122 to £5,301 depending on age and experience.

Please send applications, with curriculum vitae and the names of two referees to Dr R.M. Hicks, School of Pathology, The Middlesex Hospital Medical School, Riding House Street, London W1P 7LD, by 8 November, 1979.
3555(A)

POSTDOCTORAL RESEARCH ASSISTANT

The successful applicant will have expertise in transmission electron microscopy and a PhD in Metallurgy & Materials Science or in Physics. The project is to study (using electron microscopy; techniques to include hot-stage, X-ray analysis, high resolution and high voltage) the nucleation, microstructures and growth kinetics of intermetallic compounds in the reaction layers which grow during the fabrication and/or heat-treatment of composite A15 superconductors. There will be close collaboration, in an interactive programme, with an existing group investigating and optimising superconducting properties and with an industrial scale fabrication facility at Harwell. Prior research experience involving superconductors is not required.

Salary is on S.R.C. scales and the appointment is for three years commencing from 1st October, 1979 or as soon as possible thereafter.

Applications, together with curriculum vitae, should be sent to Dr J.E. Evetts or Dr E.R. Wallach at the above address.
3551(A)

Section Head Medicinal Chemistry

The Research & Development Division of G. D. Searle & Co., located in north suburban Chicago, has an immediate need for a Section Head within our Medicinal Chemistry Department. As principal scientific expert, leader and manager, you will assume responsibility for directing the entire Medicinal Chemistry research program in the area of gastrointestinal disease, in close cooperation with the head of gastrointestinal pharmacology. The qualified candidate will possess a Ph.D. in Chemistry plus 10 years of experience in the pharmaceutical industry, or equivalent experience in medicinal chemical research. Proven managerial skills are essential.

We offer a competitive starting salary, comprehensive benefits and the opportunity to contribute your talents to our overall research efforts.

For confidential consideration, please submit your curriculum vitae complete with salary requirements to:

L. Kedzior

Research & Development Division
G. D. Searle & Co.
P.O. Box 5110
Chicago, IL 60680

Equal Opportunity M/F/H...
A Practice, Not Just A Policy

W476(A)

SEARLE

ROYAL POSTGRADUATE MEDICAL SCHOOL

DEPARTMENT OF MEDICINE

POST-DOCTORAL IMMUNOLOGIST

required for work on the role of complement in induction of immunological responses. The project is supported by the Medical Research Council until October 1980 but there will be a continuing opportunity in the Unit for a suitable individual. For further details please contact Dr M.B. Pepys, Immunological Medicine Unit, Department of Medicine, Royal Postgraduate Medical School, 150 Du Cane Road, London W12 0HS or telephone 743 2030 extension 258.

Salary up to £5199 plus £740 London Allowance.

Application forms are available from and should be returned to the Personnel Office, Royal Postgraduate Medical School, 150 Du Cane Road, London W12 0HS quoting reference number 2/220.
3540(A)

METEOROLOGIST

This is a two-year teaching and research position in the Atmospheric Science program of the Department of Land, Air and Water Resources. The person selected will have a doctorate in meteorology or in atmospheric science or a doctorate in a related science plus professional meteorological experience. The incumbent will be expected to teach a total of three quarter courses in one or more of the areas: Atmospheric Physics, Air Pollution Meteorology, Synoptic Meteorology and Dynamic Meteorology and to support one or more of the ongoing research activities within the department. These include the study of large-scale air-sea interaction, marine and continental planetary boundary layer dynamics, and the modelling of mesoscale flows in complex terrain. Research support will be provided by the department. This position is at the Assistant Professor level and carries a 12-month salary of $19,700 and up, depending on qualifications. The position becomes available immediately, with the expectation that the person selected will be available for service on or before January 1, 1980.

Applicants should submit a vitae, statement of teaching and research experience and interests, undergraduate and graduate transcripts, list of publications, reprints, and names and addresses of at least three references, to be sent to John J. Carroll, Meteorologist Search Committee, Department of Land, Air and Water Resources, University of California, Davis, California 95616, telephone (916) 752-3245/1406. Closing date is October 15, 1979.

The University of California is an Affirmative Action/Equal Opportunity Employer.
W460(A)

KING'S COLLEGE HOSPITAL MEDICAL SCHOOL

(University of London)
Denmark Hill,
London SE5 8RX

JUNIOR MEDICAL LABORATORY SCIENTIFI OFFICER 'B'

required for the Chest Unit in this Medi School. Work will involve contact wi patients and assisting doctors with resear into diseases of the lung.

Minimum requirements, 5 'O' levels, 4 Science subjects including maths, d release available. Post suitable for sch leaver.

Salary according to age, qualificatio and experience.

Applications, in writing, giving f details should be sent to the Secretary of t Medical School at the above addre Closing date 8 November, 1979. 3541(A)

THE UNIVERSITY OF PAPUA NEW GUINEA

(Port Moresby)

Applications are invited for the post o

LECTURER GRADE 1/11 IN PHYSIOLOGY

IN THE DEPARTMENT OF HUMAN BIOLOGY

in the Faculty of Medicine. It is expec that the appointee will share in the teach of Physiology, undertake research and actively involved in the National St Development Programme. The Departm is responsible for teaching basic medi sciences to undergraduate and postgradu students. Courses offered include gr anatomy, histology, embryology, neu anatomy/neurophysiology, medical ph ology, biochemistry and pharmacology

A higher degree in physiology o medical qualification with teach experience in physiology is requir Teaching and/or research experience i developing country would be advantage. Salary scales: Lecturer Gra K11,875pa, Lecturer Grade II K13725 (£1 sterling = K1.55). Family passa baggage allowance; clinical allowanc year contract; 24% gratuity of which 5 taxable; research support; rent-f accommodation; overseas leave; educa allowances; medical scheme.

Detailed applications (2 copies) curriculum vitae and naming 3 referee be sent direct to the Secretary, Univer PO, Papua New Guinea, by 17 Decem 1979.

Applicants resident in the UK sh also send one copy to the Inter-Unive Council, 90/91 Tottenham Court R London W1P 0DT. Further details ma obtained from either address. 3544(

UNIVERSITY OF TORONTO
Scarborough College
CHEMISTRY
Assistant Professor
(tenure stream)

f inorganic, bio-inorganic or analytical hemistry from 1 July 1980. Duties include ndergraduate and graduate teaching, esearch.

Send curriculum vitae, statement of esearch interests, and names of three eferees to Professor John E. Dove, hairman, Physical Sciences Division, carborough College, University of oronto, West Hill, Ontario, Canada M1C A4.

W463(A)

THE QUEEN'S UNIVERSITY OF BELFAST
LECTURESHIP IN ZOOLOGY

Applications are invited for a post of lecturer in the department of Zoology tenable from 1st April 1980 or such other date as may be arranged. Applicants with some experience in any aspect of Cell Biology preferred but other fields of Zoology will be considered.

Initial placing, which will depend on experience and qualifications will be made at one of the first three points on the scale (under review) for lecturers: £4,335, £4,623, £4,911 rising to £8,994 with contributory pension rights under the FSSU or USS.

The appointment will be subject to a period of probation of up to three years in duration. Further particulars may be obtained from the Personnel Officer, The Queen's University of Belfast, BT7 1NN, Northern Ireland. Closing date: 30th November, 1979. (Please quote Ref. 79/N) 3581(A)

UNIVERSITI SAINS MALAYSIA
SCHOOL OF MEDICAL SCIENCES

pplications are invited for the posts of ofessor in the fields of (1) Anatomy,) Physiology and (3) Biochemistry and cturers in the fields of (1) Pathology,) Pharmacology, (3) Physiology,) Anatomy, (5) Parasitology,) Clinical Biochemistry and (7) Medical icrobiology.

Qualifications/Experience: Candidates r the post of Professor should have BBS/PhD degree with wide experience in field of teaching and research. They ould also have some outstanding public-ons to their credit. Candidates for the st of Lecturer should have MBBS/PhD gree with an interest in undergraduate ching and research. (Clinical staff will required to undertake patient care duties the Teaching Hospital and those with stgraduate specialist qualifications and ry out clinical duties will be eligible for a ecialist allowance).

Salary Range per annum: Professor: m M$35,580 to M$40,380; Lecturer: m M$15,420 to M$29,100. Other efits: return passage, excess baggage istance, paid annual leave, free medical efits for staff and family and gratuity at ½% of total monthly salary and car loan ilities. Tenure: On contract for three rs renewable if necessary.

Applications containing full personal ticulars and current salary to be warded to the Registrar, Universiti ns Malaysia, Minden, Penang, before November.

W459(A)

CHAIRPERSON OF BIOLOGY DEPARTMENT

Northeastern University invites applications for chairperson of biology, to start summer or fall 1980. Department has 21 full-time faculty and 50 more teaching, research, and support staff. It offers undergraduate major; provides courses for undergraduate professional programs; and conducts research and graduate programs in botany, zoology, microbiology, ecology, cell biology, animal physiology, and marine biology. Facilities include more than 35 laboratories, aquarium, animal rooms, animal-care center, EM center, VAX 11/780 computers with departmental terminals, vertebrate museum, herbarium, and large greenhouse. Department is affiliated with university's Marine Science Institute at Nahant. Successful candidate must have excellent research and teaching credentials; administrative experience is desired. Closing date is 30 November. Send resume with description of ongoing research, funding record, and teaching experience, with names of five references, to: **Biology Chairperson Search Committee, 403 Richards Hall, Northeastern University, Boston, Massachusetts 02115.** *Equal Opportunity/Affirmative Action/ Title IX University.* W477(A)

RESEARCH TECHNICIAN for Department of Physiology. The areas of research being renal physiology and endocrinology. The work involves a variety of biochemical assays and experience in radioimmunoassay techniques would be an advantage. Qualifications required ONC in Med. Lab. Science or equivalent. Salary Scale £3,122 to £3,553 p.a.

Application form from Assistant Secretary, Personnel Office, University of Birmingham, PO Box 363, Birmingham B15 2TT. Ref 429/c/342. 3553(A)

IMPERIAL COLLEGE UNIVERSITY OF LONDON
DEPARTMENT OF PHYSICS
LECTURESHIPS IN PHYSICS
BLACKETT LABORATORY

Applications are invited for a number of lectureships in physics from candidates whose research interests lie in the fields of astronomy, optics and laser physics, solid state physics, spectroscopy or theoretical physics.

The successful candidates will be attached to the appropriate research section within the Physics Department. Salary on the scale £4,333 to £8,992 (under review) with superannuation plus London allowance of £740 p.a.

Applications with curriculum vitae, list of publications and the names of two referees should be addressed to Professor D.J. Bradley, Department of Physics, Imperial College, London SW7 before 8th November 1979.

Applications are invited for a

POST-DOCTORAL RESEARCH ASSISTANTSHIP
OPTICS SECTION

The successful candidate will engage in a theoretical research programme in the field of laser dynamics and non-linear optics. Applicants should hold a Phd degree (or equivalent) or be in the process of completing their doctoral thesis. A reasonable background in theoretical physics is a prerequisite and previous experience in quantum electronics would be an advantage. The appointment will be for two years and the salary will be at an appropriate point on a scale starting at £4,333 (under review) plus £740 London allowance and superannuation.

Applications, accompanied by at least two references, should be sent to Dr G H C New, Optics Section, Department of Physics, Imperial College, London SW7 2BZ. 3522(A)

CSIRO AUSTRALIA
Research Scientist
Division of Mathematics & Statistics
Canberra

CSIRO has a broad charter for research into primary and secondary industry areas. The Organization has approximately 7,000 employees — 2,400 of who are research and professional scientists — located in Divisions and Sections throughout Australia.

FIELD: Applied Mathematics

GENERAL: The Division has a staff of 101 of whom seventy percent are professional scientists. Staff are located at centres and laboratories in capital cities in every state and in some major country centres.

The Division's research in mathematics and statistics includes various aspects of fluid mechanics, statistical mechanics, numerical analysis, optimization, operations research, distribution theory, multivariate analysis, stochastic processes, geostatistics, statistical inference and experimental design. Application of this research to agricultural, biological, environmental and industrial investigations, currently includes modelling of ecological, physical, agricultural, climatological systems, industrial processes and mineral surveys.

Scientists of the Division advise on the mathematical, numerical and statistical analysis of research projects undertaken by other Divisions of CSIRO.

The Division wishes to appoint a senior scientist to head a small group in applied mathematics. The present group leader, Dr J Blake, has just been appointed to the Chair of Mathematics at the University of Wollongong.

DUTIES: The successful applicant will be based in Canberra, and will be responsible for the overall direction of the group of four scientists which is represented in both Canberra and Melbourne.

The group has specific research interests in fluid mechanics, soil physics, theoretical physics in biology, applied stochastic processes and statistical mechanics.

QUALIFICATIONS: A PhD degree or equivalent qualifications with demonstrable research and leadership ability.

SALARY: Senior Research Scientist/Principal Research Scientist — $A20,198 to $A27,212 p.a.

TENURE: Indefinite with superannuation.

Applications IN DUPLICATE, stating full personal and professional details, the names and addresses of at least two professional referees, and QUOTING REFERENCE NUMBER 440/307 should reach:-

The Personnel Officer, Australian Scientific Liaison Office, Canberra House, 10-16 Maltravers Street, London WC2R 3EH, by 24th November 1979

Applications in U.S.A. and Canada should be sent to:- The Counsellor Scientific, Embassy of Australia, 1601 Massachusetts Avenue, N.W., Washington, D.C., 20036, U.S.A. 3563(A)

HOWARD UNIVERSITY
COLLEGE OF MEDICINE
FACULTY POSITION IN ANATOMY

The Department of Anatomy is seeking applications for a faculty appointment from individuals with research interest in Reproductive Biology. The candidate should have demonstrated aptitude to develop independent research programs. Applicant should have interest and experience in the teaching of either gross anatomy, histology or neuroanatomy to freshmen medical, dental and graduate students.

Applicant should send curriculum vitae and include a statement of teaching and research goals, bibliography. Reference from three individuals supporting both research and teaching capabilities should be sent to Chairman, Department of Anatomy, College of Medicine Howard University, 520 W Street, NW, Washington, DC 20059.

Howard University is an
Equal Opportunity Affirmative Action Employer

W478(A)

Northern Ireland Forensic
Science Laboratory

Document Examiner (Scientific Officer)

£3,289 – £5,085

Applications are invited for a pensionable post in the Northern Ireland Forensic Science Laboratory, Newtownbreda Road, Belfast.
The officer appointed will join a team of scientists engaged in the scientific investigations of crime throughout Northern Ireland. He/She will be involved in the comparison of handwriting and typewriting and the examination of signatures and documents to detect forgery, alterations, etc. Previous experience in this field would be an advantage.

Applicants must be under 27 years of age at 31 December 1979 and possess:

(a) a 1st or 2nd class Honours degree in a relevant scientific subject; or

(b) an equivalent qualification acceptable to the Civil Service Commissioners.

Exceptionally, applications may be considered from candidates over the age limit who have specialised experience.
Salary will be in the scale £3,289 to £5,085. The salary scale with effect from 1 January 1980 will be £3,591 to £5,486. Starting salary will be related to qualifications and experience.

There are promotion prospects to Higher Scientific Officer £4,727 to £6,211 (£5,097 to £6,737 from 1 January 1980), Senior Scientific Officer £5,876 to £7,864 (£6,332 to £8,705 from 1 January 1980) and Principal Scientific Officer £7,534 to £9,646 (£8,615 to £11,343 from 1 January 1980).

Please write or telephone for an application form quoting job reference SB 362/79/NA to the Civil Service Commission, Rosepark House, Upper Newtownards Road, Belfast BT4 3NR (telephone Dundonald 4585 ext 256). Completed forms must be returned to arrive not later than 8th November, 1979. 3552(A)

English
China
Clays
group

RUBBER TECHNOLOGIST

English Clays Lovering Pochin & Co Ltd, the major operating Company of the English China Clays Group has a vacancy for a Rubber Technologist within its Research and Development Department.

The job involves providing a technical advice service, promoting the Company's products to the rubber industry and maintaining and developing technical contacts with the market. The job holder will also be responsible for specific projects associated with developments in rubber technology.

Candidates should possess a minimum of an HNC and will have at least five years experience of rubber technology. Experience of the rubber markets in Western Europe and extender technology related to the Company's mineral products is also desirable.

The position is based at the Company's well equipped Laboratories at St Austell in Cornwall where facilities provided are excellent.

Competitive salary is negotiable and fringe benefits include Profit Participation, Pension and free Life Assurance, full assistance will be given with re-location expenses.

Applications, together with a curriculum vitae, quoting reference 285B, to: The Personnel Manager, John Keay House, St Austell, Cornwall.

3557(A)

UNIVERSITY OF VICTORIA
Victoria, British Columbia, Canada

DEPARTMENT OF BIOCHEMISTRY AND MICROBIOLOGY:

Applications are invited for the position of

ASSISTANT PROFESSOR

beginning 1 July, 1980. Candidates should have strong teaching and research interests in one or more of the following fields: protein biochemistry, molecular genetics (recombinant DNA), eukaryotic cell membrane receptors. The successful applicant will be required to teach biochemistry at the undergraduate and graduate levels and carry on an active research program. A PhD is required and postdoctorate experience desirable.

Applications with a curriculum vitae, list of publications, a brief statement of research plans, and the names of three references who have been requested to send letters of recommendation should be sent to Prof. A.T. Matheson, Chairman, Department of Biochemistry and Microbiology, University of Victoria, PO Box 1700, Victoria, British Columbia, Canada V8W 2Y2, as soon as possible.
W462(A)

DEPARTMENT OF ANTHROPOLOGY University of Alberta. Position vacancy. The department of Anthropology, University of Alberta, is seeking to hire a physical anthropologist at the level of Assistant Professor. The position is tenure-track and begins July 1, 1980. The present salary is $19,700.00 per annum (subject to 1980 negotiations and can be adjusted upwards according to credentials). PhD required or to be completed by September, 1980. Must be able to teach first-year general anthropology and introductory and advanced courses in physical anthropology, with specialization in human evolution and human osteology. Additional qualifications in comparative osteology and/or human biology sought. The University of Alberta is an equal opportunity employer. Deadline for applications March 1, 1980. Send vitae and names of referees to Dr C.G. Hickey, Chairman, Department of Anthropology, Edmonton, Alberta, Canada T6G 2H4.
W461(A)

MISSISSIPPI STATE UNIVERSITY
DEPARTMENT OF ENTOMOLOGY
ASSOCIATE FULL PROFESSOR

Applications are invited for a full time (12 mos.) appointment with rank of Associate or Full Professor in the Department of Entomology, Mississippi State University. The successful candidate will be responsible for research in pest management on field crop systems with particular emphasis on cotton and soybean insect problems, and for participation in ongoing interdisciplinary research to develop and apply insect-crop pest models and management systems. Teaching and guidance of graduate research will be expected in areas of insect ecology and/or population dynamics. PhD in entomology or relevant life science and experience in field crop research are required.

Experience in modelling and computer programming is desirable. Salary is negotiable and competitive. Application deadline: December 15, 1979. Job available January 1, 1980.

Send resume, list of publications and 3 letters of reference to: Dr E.P. Pieters, Chairman, Search Comm., Entomology Department, Drawer EM, Mississippi State, MS 39762. Mississippi State University is an Affirmative Action/Equal Opportunity Employer. W474(A)

CENTRAL PUBLIC HEALTH LABORATORY
National Collection of Type Cultures
MEDICAL LABORATORY SCIENTIFIC OFFICER
or
SENIOR MEDICAL LABORATORY SCIENTIFIC OFFICER

MLSO with minimum 4 years experienc
post-AIMLS or equivalent or a Senio
MLSO (recently qualified FIMLS) i
required for the National Collection o
Type Cultures. Duties will includ
maintenance and testing of Collectio
strains, examination of strains sent fc
identification, and opportunity to assist i
research programmes. An interest i
bacterial classification and identificatio
would be an advantage. Experience will b
gained with a wide range of bacteri
Further details and application form
available from:

The Personnel Officer, Central Publi
Health Laboratory, Colindale Avenu
London NW9 5HT. Salary MLSO scal
£3768 to £5391 plus £354 Londo
Weighting. Snr MLSO scale £5016 to £658
plus £354 London Weighting. 3538(A)

RADIOBIOLOGIST (Basic Grad
Belvidere Hospital, London Roa
Glasgow G31 4PG. Applications a
invited for the above post in the Radi
biology Research Group of the Glasgo
Institute of Radiotherapeutics an
Oncology. Research activities of the Grou
include the responses of normal an
tumour tissue to X- and neutron rad
ations, hyperthermia and chemother
peutic agents, both 'in vitro' and in mic
Candidates should have a good honou
degree, preferably in a biological scienc
Experience in radiobiology and in handli
small animals would be an advantag
Salary dependent on qualifications an
experience, ranges from £3,486 to £5,5
(from 1.1.80, £3,807 to £6,075
Application form and further informati
can be obtained from Dr. J. Kir
Radiobiology Research Group, at t
above address. Telephone 041-554 185
extension 383. Closing date for appl
cations is Monday, 12th November, 197
3547(A)

CHARING CROSS HOSPITAL MEDICAL SCHOOL
(University of London)
LECTURESHIP IN PHYSIOLOGY

Applications are invited for a Lectures
in Physiology to start as soon as possi
after April 1980. The successful candid
will be expected to participate in
teaching of 1st, 2nd and 3rd year und
graduate preclinical medical students a
to maintain an active research program
Currently the Department has resea
interests in microcirculation, cardiac a
skeletal muscle, kidney, endocrines a
neurophysiology. There are excell
library and research facilities and ther
extensive inter-departmental collab
ation.

Salary on Scale £4,333 to £8,992 + £
London Weighting p.a. according to
and experience.

Arrangements to visit the Departm
should be made with the Head
Department, Dr .L.H. Smaje (Tel: 01-
2040 Ext. 2850) from whom further det
may be obtained.

Applications including a curricul
vitae and the names of 2 referees should
sent to The School Secretary, Char
Cross Hospital Medical School,
Reynolds Building, St. Dunstan's Ro
London W6 8RP by 30th November. (R
074/5) 3578(A

Product Manager-Medical Equipment

Essex to £7,000+car.

My client markets one of the most comprehensive ranges of medical and hospital equipment in the world. Their products are universally respected for their fine design and reliability. The key role of Product Manager for their Intensive Care products provides the link between user and product development, and between sales and manufacturing.

You'll be responsible for keeping the existing product range competitive by close liaison with the medical profession, identifying the need for new products. Short and long term market and production planning is a further important area, as is the training of the sales force and distributors.

We are looking for a mature and level headed man or woman with a solid marketing background in the fields of pharmaceuticals, medical equipment or hospital services. A degree in Science, Engineering or Business Studies would be appropriate, but your experience of modern marketing techniques in a profit conscious organisation is more important. You should be at least 27, and ideally in your early to mid-thirties.

The organisation should have no problems in satisfying your future development needs, and the employment package is realistic, with excellent relocation assistance where appropriate to this relatively low cost housing area.

If you think you fit the bill, talk things over with me, David Woolf on 01-262 0184 or 0376 513513.

Alternatively, send me a brief c.v. to Macmillan Woolf, Personnel Consultants,
North West House, 119/127 Marylebone Road,
London NW1 5PU

Macmillan Woolf
Personnel Consultants

3564(A)

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF VETERINARY PATHOLOGY

Applications are invited from holders of good honours degrees in Biochemistry or an allied subject, for the post of Research Assistant/Senior Research Assistant in the Department of Veterinary Pathology. The project is concerned with the intracellular localisation of copper and the isolation of copper binding proteins in chronic copper poisoning. The successful applicant will be eligible to register for a higher degree.

The project is financed for two years in the first instance. The initial salary will be within the range £3,776 to £4,910 per annum.

Applications, together with the names of three referees, should be received not later than 15th November, 1979, by The Registrar, The University, PO Box 147, Liverpool L69 3BX, from whom further particulars may be obtained. Quote Ref: RV/824. 3572(A)

UNIVERSITY OF HONG KONG

LECTURESHIPS IN PATHOLOGY

Applications are invited for two posts of Lecturer in Pathology. Applicants must hold primary medical or dental qualifications. Possession of an approved higher qualification in Pathology and appropriate experience in the speciality is required for appointment above the bar on the salary scale. Starting salary will depend on qualifications and experience.

Annual salary (superannuable) is: HK$68,580 — 72,060 — 83,340 — 87,240 — 91,320 — BAR 109,920 — 114,840 — 119,760 — 124,680 — 129,600. (£1 = HK$10.70 approx).

At current rates, salaries tax does not exceed 15% of gross income. Housing at a rental of 7½% of salary, education allowances, long leave and medical benefits are provided.

Further particulars and application forms may be obtained from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H OPF, or the Assistant Secretary (Recruitment), University of Hong Kong, Hong Kong.

The closing date for applications is *30th November 1979*. 3561(A)

FACULTY POSITION THEORETICAL NUCLEAR PHYSICS

Indiana University Bloomington

The Department invites applications for appointment as a senior nuclear theorist at the Professor or Associate Professor level with tenure. Candidates should have demonstrated originality and excellence in research. Preference will be given to applicants with the interest and ability to interact with the present theoretical and experimental nuclear physics programs at Indiana University, particularly the intermediate-energy research programs of the Indiana University Cyclotron Facility.

Interested persons should contact Professor Roger G Newton, Chairman, Department of Physics, Indiana University, Bloomington, Indiana 47405. Indiana University is an Affirmative Action/Equal Opportunity Employer. Deadline for applications February 1, 1980. W475(A)

THE UNIVERSITY OF PAPUA NEW GUINEA

(Port Moresby)

Applications are invited for the post of

LECTURER/SENIOR TUTOR IN SCIENCE

Goroka Teachers College

The appointee would be responsible for teaching in two of the following areas: Physics, Geology or Chemistry in the Science Department and would also be involved in some general science teaching. Applicants should hold an appropriate degree and a teaching qualification. Salary Scales: Lecturer K13300 pa, Senior Tutor K11450 pa, (£1 sterling = K1.55). Family passages, baggage allowance; 3 year contract; 24% gratuity of which 5% is taxable; research support; overseas leave; education allowances; medical scheme.

Detailed applications (2 copies) with curriculum vitae and naming 3 referees to be sent direct to the Secretary, Box 4820, University PO, Papua New Guinea by 14 November 1979.

Applicants resident in the UK should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address. 3542(A)

University of Petroleum and Minerals

Dhahran, Saudi Arabia

Crystallographer

A Crystallographer is sought to work as a research associate with a distinguished Chemistry Professor on the structure of organic phosphorus compounds. Applicants should have a Phd plus some experience in chemical crystallography and should also have programming knowledge. Facilities available include a CAD-4 Automatic Diffractometer with a PDP 8/A Computer, a Weissenberg Camera.

Minimum regular contract for two years, renewable. Competitive salaries and allowances. Air conditioned and furnished housing provided. Free air transportation to and from Dhahran each two-year tour. Attractive educational assistance grants for school-age dependent children. All earned income without Saudi taxes. Ten months duty each year with two months vacation paid.

Apply with complete resume on academic and professional background, indicating home and office addresses, telephone numbers and date of availability for employment, together with three references and with copies of degrees and transcripts to:

Dean of Faculty and Personnel Affairs,
University of Petroleum and Minerals,
PO Box 144, Dhahran, Saudi Arabia.

3548(A)

Research Opportunities in Pharmacology/ Biochemistry

As a result of the recent expansion of our R&D facilities two new posts are available in the Pharmacology Department at the Wyeth Institute of Medical Research, Taplow, near Maidenhead. The successful candidates will join a team of scientists working to define the actions of drugs in mental disorders. One of the applicants will contribute to these studies by assisting in the design and application of biochemical techniques for defining the mechanics by which drugs act within the central nervous system. The other will assist in the development and use of behavioural tests for investigating psychoactive drugs.

The new posts will be of interest to recent graduates with good honours degrees in biochemistry or pharmacology and to those who have some special training in the techniques of psychopharmacology.

The modern laboratories are situated in a pleasant rural setting in Berkshire.

Applicants should send a brief but comprehensive curriculum vitae to:

Miss C. Bradshaw, Personnel Officer,
Wyeth Laboratories, Huntercombe Lane
South, Taplow, Maidenhead, Berkshire
SL6 0PH. Tel. No. Slough 28311.

Wyeth Laboratories 3585(A)

HEAD TECHNICIAN

£6,597 – £8,691

Department of Viral Products

The Institute's main functions are the standardization and control of biological substances used in human medicine, work which is well supported by research. The Division of Viral Products is a large and active division responsible for research and development work in the control of viral vaccines and associated biological products used in man.

This is an important post which calls for considerable experience in microbiology, general laboratory management, and overall supervision of assistant staff. Previous experience in virology and the safe handling of potentially pathogenic organisms would be an advantage.

Salary on Chief or Senior Chief Scale dependant on qualifications and experience within the range: £6,597 to £8,691.

**Further details and application forms are available from the Personnel Officer, National Institute for Biological Standards and Control, Holly Hill,
Hampstead, London
NW3.
Telephone 01-435-2232
Extension 200, quoting
reference VP/112.**

NIBSC

National Institute for Biological Standards and Control

3594(A)

IMPERIAL CANCER RESEARCH FUND

TECHNICIAN/ RESEARCH OFFICER

1. to assist our Epithelial Cell Growth Regulation Laboratory with work on the biology of breast cancer and related projects. Experience of tissue culture and biochemical techniques desirable. Reference ECG1.
2. to assist our Cell Differentiation Laboratory at Mill Hill, London NW7, with the biochemical analysis of cell differentiation in the cellular slime mould Dictyostelium discoideum. Experience in enzyme assay and analytical techniques preferred. Reference MH1.

HNC or Degree. Salary range £4,122 to £5,950. Four weeks holiday. Pension Scheme. For further information and application form write or telephone to Miss S.M. Hurley, Imperial Cancer Research Fund, Lincoln's Inn Fields, London W.C.2. on 242 0200 ext. 305, quoting appropriate reference.

3567(A)

THE MACAULAY INSTITUTE FOR SOIL RESEARCH

DEPARTMENT OF SOIL FERTILITY

Applications are invited for an appointment of a

SCIENTIFIC OFFICER/ HIGHER SCIENTIFIC OFFICER

to engage in field experiment/advisory work in relation to soil productivity and crop nutrition, including trace element and other mineral element status and to participate in collaborative work with the Departments of the Institute, *viz.* Soil Survey (soil series information), Spectrochemistry (trace element work), Statistics (soil data bank). Candidates should have a First or Upper Second Class Honours Degree and preferably a post-graduate degree in a relevant subject such as Soil Science or other equivalent experience, particularly in relation to the practical application of agricultural work. An interest in computing and statistics would be welcome. The Department is well equipped for the work and has call upon the trace element analysis service of the Department of Spectrochemistry.

Salary £3,289 to £5,285 (Scientific Officer), £4,727 to £6,211 (Higher Scientific Officer) per annum according to qualifications and experience: two years' relevant post graduate experience is required for Higher Scientific Officer appointment. Non-contributory superannuation scheme operative.

Forms of application and further information may be obtained from The Secretary, The Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen, AB9 2QJ, to whom they should be returned by 9 November 1979. Quote Ref. 79/32

3569(A)

INTERFERON PRODUCTION

BIOCHEMISTRY/ VIROLOGY PhD

or equivalent required to head small established group involved in human leukocyte interferon production. This project is a joint effort of the Memorial Sloan-Kettering Cancer Center, New York, and the University of Berne. The successful applicant will be appointed to an appropriate position within the MSKCC.

The appointment will be initially for one year, to start as soon as possible. Salary and conditions of employment are those of the Canton of Berne.

Applications, with curriculum vitae and the names of two referees, should be sent to Dr V.G. Edy, Theodor Kocher Institute, Postfach 99, 3000 Berne 9, Switzerland.

W457(A)

UNIVERSITIES OF BRISTOL AND MANCHESTER

DARESBURY LABORATORY

POSTDOCTORAL RESEARCH ASSOCIATE

Applications are invited for a postdoctoral research associate to coordinate an SRC collaborative computational project on molecular collisions. The project will involve the development of computer programmes for use at Daresbury and other centres. The post is based either at the Department of Chemistry, University of Manchester under Dr J.N.L. Connor or at the Department of Chemistry, University of Bristol, under Dr G.G. Balint Kurti or Dr J. Gerratt.

The location of the post will depend on the field of interest of the successful candidate. The project will be carried out in collaboration with Professor P.G. Burke's group at Daresbury Laboratory.

Salary within the range £4,333 to £5,199 pa. Superannuation.

Applications with curriculum vitae and the names of two referees to Dr J.N.L. Connor, Department of Chemistry, University of Manchester, Manchester M13 9PL by December 1st, 1979.

3583(A)

CERRO TOLOLO INTER-AMERICAN OBSERVATORY

EXPERIMENTAL PHYSICISTS OR ASTRONOMERS

The observatory (operated by the Association of Universities for Research in Astronomy, Inc. in Tucson, Arizona under contract with the U.S. Nationa Science Foundation) has staff positions available for experimental physicists or astronomers. The ideal candidates will have strong backgrounds in detector technology, low noise solid state electronics, cryogenic techniques and preferably advanced degrees and job related experience.

The selected candidates will have responsibility for the design and development of detector systems and associated hardware for the observatory' astronomical infrared research programs.

Salaries and position levels negotiabl according to qualifications.

The principal work location is in the coastal city of La Serena, Chile. Libera fringe benefits, including oversea allowance, observatory provide reasonable rent housing close to work, a well as opportunities to participate in th observatory's research programs.

Candidate selections will be made o January 31 and June 30, 1980. Sen Resume to: Personnel Office, 950 N Cherry Ave., P.O. Box 26732, Tucson, A 85726

W447(A)

THE UNIVERSITY OF BRITISH COLUMBIA

DEPARTMENT OF ZOOLOGY

plications are invited for the position of

ASSISTANT PROFESSOR

the Department of Zoology, University
British Columbia. Qualifications
lude a PhD in biology or a related field.
The successful applicant will be expected
teach and develop a strong experimental
earch programme in invertebrate
bryology and morphogenesis. Upper
el undergraduate teaching, and graduate
dent teaching and supervision is
uired.
Salary will be commensurate with
perience and duties. Curriculum vitae
d the names of 3 referees should be
mitted to Dr G.G.E. Scudder, Head,
partment of Zoology, University of
tish Columbia, Biological Sciences
ilding, 6270 University Boulevard,
ncouver, B.C. Canada V6T 1W5.
The expected date of appointment is July
1980, subject to budgetary approval.
sing date for application is when
ition is filled. W469(A)

UNIVERSITY OF WAIKATO

New Zealand

JUNIOR LECTURER/ ECTURER IN CHEMISTRY

(A79/26)

University of Waikato has a current
ancy for a Junior Lecturer or Lecturer,
ferably with teaching interests in
sical chemistry. Chemistry is offered
in the School of Science which
ourages research in applied environ-
ntal and interdisciplinary fields. The
ointment may be made for a limited
n.
he current salary scales in New Zealand
ersities cover the following ranges:
ior Lecturers NZ$10,016 to $12,298
Lecturers NZ$13,722 to $16,861 p.a.
ethod of application and conditions of
ointment are available from the
strar, University of Waikato, Private
Hamilton, New Zealand, or from the
ociation of Commonwealth
ersities (Appts), 36 Gordon Square,
don WC1H 0PF.
pplications close on *10 December 1979.*
3560(A)

TANFORD UNIVERSITY

GEOCHEMIST —PETROLOGIST

Department of Geology in the School
arth Sciences invites applications for a
lty position beginning September,
. Areas of interest include (but are not
ed to) geochemical processes in the
and mantle of the earth including
pe geochemistry, thermodynamics,
tics, element distribution and phase
ibria studies. Teaching responsibilities
t include graduate and undergraduate
ses in geochemistry and igneous
logy. Level of appointment is open,
we are particularly interested in a
ive young person, with a well-
lished record in research.
plications should include (1) a resume
ucation, professional experience, and
shed research; (2) a brief statement of
for future research, and (3) the names
referees. Send to: Prof. J.G. Liou,
artment of Geology, Stanford
ersity, Stanford, CA 94305, by
mber 1st, 1979. Stanford University is
ual-opportunity/affirmative-action
oyer. W329(A)

UNIVERSITY OF CALIFORNIA, BERKELEY

DEPARTMENT OF ZOOLOGY

POSITION IN DEVELOPMENTAL BIOLOGY

Applications are invited for an anticipated opening at the level of Assistant Professor of Zoology pending final budgetary approval. The teaching and research interests of the candidate should be in the area of animal developmental biology with emphasis on mechanisms operating at the cell and tissue level. Possible areas of specialization may include, but are not restricted to, gene expression in development, mechanisms of morpho-genesis, regulation of pattern formation, and developmental neurobiology.

Teaching responsibilities may include an advanced undergraduate course in developmental mechanisms, a graduate speciality course, and contributions to the teaching of general biology.

Applicants should send a curriculum vitae, bibliography with copies of publications, a summary of research and teaching objectives, and the names of three references by January 1, 1980 to Chairman, Search Committee in Development, Department of Zoology, University of California, Berkeley, California 94720.

The University of California is an Affirmative Action Employer. W470(A)

LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE

(University of London)

Keppel Street, WC1E 7HT

DEPARTMENT OF MEDICAL MICROBIOLOGY

POSTDOCTORAL RESEARCH FELLOW

Applications are invited for a post tenable up to 30 April 1981 to work in collaboration with Dr C R Howard and Dr D I H Simpson on the structure and replication of Pichinde virus. Previous experience in the field of biochemical analysis of viral nucleic acids would be advantageous.

Appointment will be made on the grade 1A for university research staff, £4,333 to £7,521 + £740 London Allowance, subject to review).

Applications, consisting of full curriculum vitae and naming two referees, should be sent to F.C. Ball, Assistant Secretary. 3588(A)

WASHINGTON UNIVERSITY SCHOOL OF MEDICINE

Assistant Professor of Microbiology

The Department of Microbiology and Immunology, Washington University, School of Medicine invites applications from highly qualified individuals for an appointment as Assistant Professor.

Applicants should have PhD or MD degrees, 2-5 years of post-doctoral research experience and a commitment to medical and graduate student teaching. Teaching competence should be in pathogenic microbiology; research interests should be in microbiology.

Send curriculum vitae, brief description of research plans and names of three referees to Dr Jospeph M. Davie, Department of Microbiology and Immunology, Washington University School of Medicine, St. Louis, MO 63110 by Nov.1, 1979. Equal Opportunity and Affirmative Action Employer M/F/H.
W401(A)

SANDOZ

Pathology

Sandoz Limited is a major Swiss-based multinational pharmaceutical company with a large, modern and well equipped Toxicology Institute in Basle, Switzerland. We are looking for suitable candidates to fill 2 posts that have recently become vacant in our Pathology Department.

A. Head of Section

Job Description: Overall responsibility for the activities of the Pathology Section which comprises a staff of about 20 members including 4 pathologists (Medical Doctors and Veterinarians). The routine activities of the section include the planning, supervision, evaluation and reporting of the post mortem aspects of chronic toxicity studies conducted on animals, including carcinogenicity tests. Since academic members of the Department are also encouraged to pursue their own research interest and to investigate the mechanisms by which drugs induce their toxicity, the Section Head is also expected to be in a position to contribute and encourage such activities. There will be ample opportunities to attend scientific conferences. The Head of Pathology is directly responsible to the Head of Toxicology.

The Ideal Candidate: Will be 30-45 years of age, possess a medical or veterinary qualification (preferably with a higher degree in Pathology) and will have considerable practical experience of laboratory animal pathology. Administrative ability is required for this position as is excellent written and spoken English. Previous industrial experience and a knowledge of German would be advantageous.

B. Pathologist

Job Description: The planning, supervision, evaluation and reporting of the post mortem aspects of chronic toxicity studies conducted on animals (especially carcinogenicity tests on rats and mice). In addition, the investigation of the mechanism by which drugs induce their toxicity. There will be ample opportunities to pursue individual scientific interests, to attend scientific conferences and for the periodic updating of expertise through stays in external laboratories (universities, etc). The pathologist will have 2 histology laboratories and attendant staff (5 technicians) at his disposal. He will be directly responsible to the Head of Pathology.

The Ideal Candidate: Will be 30-45 years of age and have a degree in Medicine, Veterinary Medicine or an appropriate biological discipline. In addition, he will have experience of laboratory animal pathology, preferably with specialized knowledge of tumor pathology and its diagnosis. Organizational talent is required for this post and previous industrial experience and some knowledge of German would be advantageous.

General Information:

- Removal expenses to Switzerland will be defrayed by Sandoz Ltd.
- The firm runs a contributory pension scheme.
- The Toxicology Institute is located in pleasant rural surroundings some 6 miles from the centre of Basle.

Interviews of suitable candidates for both posts will be held in London in mid-November. Short list candidates will be subsequently invited to Basle.

Applications, which should be made in writing, should be accompanied by a comprehensive curriculum vitae and the names of two referees.

Apply to: The Personnel Department, Ref. 1244, Sandoz Ltd., CH-4002 Basle, Switzerland.

W458(A)

INSTITUTE OF OCEANOGRAPHIC SCIENCES

HIGHER SCIENTIFIC OFFICER/SENIOR SCIENTIFIC OFFICER/PRINCIPAL SCIENTIFIC OFFICER

The Institute has vacancies for a Continental Margin (post 1) and an Experienced Geochemist (post 2).

Post 1 Continental Margin Geologist

The Institute is carrying out research into the structure and evolution of continental margins, both adjacent to the British Isles and elsewhere. The research programme includes the acquisition of geological and geophysical data at sea, the compilation of data from other sources, and the analysis and interpretation of these data. In addition to improving our understanding of the nature of passive continental margins and subsided microcontinents, the data are used to provide advice to government on the geological and geophysical criteria with which continental margins can be defined in the context both of existing and proposed International Law.

The successful candidate will be a geologist or geophysicist familiar with marine geophysical techniques. Additional experience in structural and stratigraphic geology, sedimentology or igneous petrology would be advantageous. The job will involve going to sea on research ships for a month or two each year.

Post 2 Geochemist

The vacancy is in a group engaged in a study of geochemical cycling in the marine environment. The programme includes studies of pore water chemistry, development and use of coring techniques for the collection of minimally disturbed cores in the deep ocean, major and minor element analyses and mineralogical examination of cores from a range of oceanographic areas. Additional work with the objective of assessing the role of sediment components in controlling the migration of heavy metals and actinides in ocean sediments is planned.

An experienced geochemist is required to take charge of the sediment geochemistry aspects of this programme. Several years postgraduate experience in marine geochemistry, including collection of deep-sea cores and chemical and mineralogical analysis, are essential.

The appointments will be as a:-

Higher Scientific Officer (HSO) or Senior Scientific Officer (SSO) for post 1 and Senior Scientific Officer (SSO) or Principal Scientific Officer for post 2 on the following salary scales:

HSO —	present rates	£4,727 to £ 6,211 pa	with 22 days paid
	from 1.1.80	£5,097 to £ 6,737 pa	annual leave
SSO —	present rates	£5,876 to £ 7,864 pa	with 22 days paid
	from 1.1.80	£6,332 to £ 8,705 pa	annual leave
PSO —	present scale	£7,534 to £ 9,646 pa	with 5 weeks paid
	from 1.1.80	£8,613 to £11,343 pa	annual leave

Starting salary may be above the minimum according to qualifications and experience. There is a non-contributory pension scheme.

The Natural Environment Research Council is not a government department but conditions of service are similar to those of the Civil Service.

Applications forms and further details from:- The Secretary, Institute of Oceanographic Sciences, Brook Road, Wormley, Godalming, Surrey, GU8 5UB. Tel: Wormley (042-879) 4141, Ext 272. Please quote reference 79/006/458 for post 1 and reference 79/010/540 for post 2.

Closing Date: 16.11.79. 3543(A)

NEUROBIOLOGY RESEARCH ASSOCIATE

To participate in studies of cellular events in the formation, maintenance, and elimination of nerve-muscle synapses, using primarily electrophysiological and E.M. techniques.

Reply to Dr W.J. Betz, Dept. Physiology, University of Colorado School of Medicine, Denver, CO80262, USA.

The University is an equal opportunity/affirmative action employer. W471(A)

UNIVERSITY OF CAMBRIDGE

CAVENDISH LABORATORY

RESEARCH ASSISTANT in the Theory of Condensed Matter Research Group

Applications are invited for the post of Research Assistant to work in collaboration with Dr J.C. Inkson and Dr M. Pepper on the physics of low density one and two dimensional electron gases in semiconductors. The post is intended for a theoretician who can interact strongly with experimentalists in what is a rapidly developing field.

This project is supported by the SRC. The initial appointment will be for two years and the salary will be in a range to £6,066 p.a. depending upon age and experience. Applications with the names of two referees should be sent before 22 November to The Secretary, Cavendish Laboratory, Madingley Road, Cambridge CB3 0HE from whom further details can also be obtained. 3592(A)

TECHNICIAN for Department of Zoology for ODA Tsetse Fly Project. Duties include routine maintenance of cultures and host animals in addition to general assistance with behavioural experiments. Salary scale £3122 to £3553 p.a. Application form from Assistant Secretary, Personnel Office, University of Birmingham, PO Box 363, Birmingham B15 2TT. Ref 141/C/385. 3546(A)

MICHIGAN STATE UNIVERSITY

POSTDOCTORAL RESEARCH POSITIONS in Environmental Carcinogenesis-Mutagenesis

Openings for research associates with strong background in molecular biology, biochemistry, microbiology, or biophysics to study the relationship between DNA repair, mutagenesis, and oncogenic transformation of human cells in culture by physical and chemical carcinogenic agents. One year with option for renewal for additional years with mutual agreement. Send application, including curriculum vitae, reprints, and letters of recommendation to:

Drs Veronica M. Maher or J. Justin McCormick Co-Directors Carcinogenesis Laboratory-Fee Hall, Michigan State University, East Lansing, MI.48824.

An affirmative action/equal opportunity employer. W467(A)

Immunoparasitologist

Assistant to Associate Professor

WASHINGTON STATE UNIVERSITY

Immunoparasitologist for research and teaching. Opportunities for advancement and tenure consideration after appropriate service. Salary — competitive and dependent upon qualifications. Requirements — PhD and post-doctoral experience which includes training in both immunology and parasitology. An opportunity for an industrious scientist to join a progressive interdisciplinary department.

Contact Robert B. Wilson, Head, Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, Washington 99164. Washington State University is an equal opportunity, affirmative action employer.

In order to assist our efforts to meet affirmative action goals, minority, handicapped, and women candidates are encouraged to identify themselves in their applications. Applications should be received by February 1, 1980.

W472(A)

UNIVERSITY OF TORONTO

SCARBOROUGH COLLEGE

Canada

MATHEMATICS ASSISTANT PROFESSOR

(tenure stream) appointment commencing July 1, 1980. Duties include undergraduate teaching, graduate teaching (at the University's main campus), and research.

Send curriculum vitae including a statement of current research activity and names of three referees to:
Professor John E. Dove, Chairman, Physical Sciences Division, Scarborough College, University of Toronto, West Hill, Ontario, Canada, M1C 1A4. W464(A)

UNIVERSITY OF BRADFORD

LECTURER IN PHYSICS

Applications are invited from suitably qualified physicists for the permanent post of Lecturer in Physics in the School of Applied Physics.

The research interests of the School are in Nuclear Structure Physics and Physical Electronics and an appointment will not be made for research in other areas of Physics.

Salary within the range £4,333 to £8,992 per annum (under review). Further particulars and application forms (to be returned by 30 November 1979) obtainable from the Registrar, Post Ref: PY/L/7X. University of Bradford BD7 1DP.

3565(A)

ANIMAL HEALTH TRUST

SMALL ANIMAL CENTRE

RESEARCH ASSISTANT

Applications are invited from veterinary graduates or other suitably qualified persons for the post of Research Assistant to study the pathology and pathogenesis of duck virus hepatitis and other related diseases of the duck. Field work will be included in this study.

The successful applicant, who will preferably have some experience of avian pathology, will join a team investigating virological and serological aspects of these diseases. Encouragement will be given to register for a higher degree if appropriate.

The initial appointment will be for three years. Starting salary will be on the scale £4,910 to £7,521 p.a. (with USS) according to age and experience.

For further information please contact Dr P.R. Woolcock at the Animal Health Trust, Small Animals Centre, Lanwades Park, Kennett, Newmarket, Suffolk. CB8 7PN. Telephone Newmarket 750543 (0638). Applications to include full curriculum vitae and the names of two referees to be sent to The Director, Animal Health Trust, Lanwades Hall, Kennett, Newmarket, CB8 7PN by November 30th 1979. 3568(A)

PROCESSORS & GROWERS RESEARCH ORGANISATION (PGRO)

Thornhaugh, Peterborough, PE8 6HJ

TECHNICAL OFFICER (A)

Applications are invited from suitab qualified young persons for the above pos which involves responsibility for running small processing laboratory, and allie activities, including involvement in fiel trials of vegetables for canning and quick freezing.

Full details, including job descriptio conditions of employment and applicatio form available on request from th Director. 3570(A)

ST. GEORGE'S HOSPITAL MEDICAL SCHOOL (University of London)

TECHNICIAN 5 (IMMUNOLOGY)

required initially to assist in a project und Dr B. Anderton involving the isolation brain proteins and the production monoclonal antibodies to those proteins.

HNC/degree and experience in prote chemistry, or immunochemistry or tissu culture essential. The appointment permanent and pensionable. Emolumen £3,700 to £4,320 (under review) plus £3 p.a. London allowance.

Application forms and further partic lars from the Establishment Office St George's Hospital Medical Schoo Cranmer Terrace, London SW17 0R Closing date 19th November. 3587(A)

UNIVERSITY OF OXFORD

DEPARTMENT OF BIOCHEMISTRY

DEPARTMENTAL DEMONSTRATORSHIP

It is proposed to appoint a Departmen Demonstrator as soon as possible. T salary will be on the scale £3,775 to £5,0 p.a., according to qualifications a experience and with membership of U.S. The appointment is for a period n exceeding 3 years. The successful candida will be expected to take part in depa mental teaching and to conduct his or h own research.

Applications (3 copies) including t names of two referees, should be sent n later than 30th November, 1979 to:

The Administrator, Department Biochemistry, South Parks Road, Oxfo OX1 3QU. 3532(A)

AN FORAS TALUNTAIS

invites applications for a

RESEARCH POST IN REPRODUCTIVE ENDOCRINOLOGY

or a related discipline. The appointment will be for a period of not less than two years and will involve research on the endocrine cause of anoestrus and embryonic mortality in beef cows.

ESSENTIAL: An Honours University Degree or equivalent in a relevant discipline. *DESIRABLE:* (i) a PhD in Reproductive Endocrinology or in a related discipline, (ii) experience of hormone assay methodology.

Application form and further particulars may be obtained from the Personnel Department (ref. R.S.), An Foras Taluntais, Headquarters, 19 Sandymount Avenue, Ballsbridge, Dublin 4, Ireland. Latest date for receipt of completed forms Friday, 30th November, 1979. 3577(A)

CHURCHILL HOSPITAL RESEARCH INSTITUTE

(University of Oxford)

Applications are invited for a BIOLOGIST

o join a research team investigating the ffects of therapeutic doses of x-rays and ast neutrons on normal tissues. The uccessful candidate will be expected to articipate in studies aimed at examining oth physiological and histological hanges in tissues after irradiation. The ost, which is grant aided, is suitable for omeone with a higher degree or equivalent esearch experience.

Further information and applications, ith the names of two referees by 16 ovember, to Dr J.W. Hopewell, hurchill Hospital Research Institute, eadington, Oxford. 3549(A)

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF VETERINARY PATHOLOGY

pplications are invited for the post of ecturer in the Department of Veterinary athology.

Preference will be given to veterinary raduates with interests and training in linical Chemistry, but applications are lso invited from biochemists or recent eterinary graduates contemplating a areer in this field.

The salary scale is £4,333 to £8,992 per nnum.

Applications, together with the names of ree referees, should be received not later an 15 December 1979 by The Registrar, he University, PO Box 147, Liverpool 69 3BX, from whom further particulars ay be obtained. Quote ref: RV/825. 3571(A)

UNIVERSITY OF SOUTHAMPTON

OUTHAMPTON MEDICAL SCHOOL

POSTDOCTORAL SCIENTIST

r a post funded by an MRC Programme rant to Professors G.T. Stevenson and .H. Wright. The work involves immunoemical studies of human lymphoma, ith particular regard to surface immunoobulins and lysosomal enzymes.

Appointment for 5 years in the first stance, on University Research Scale 1A: ,333 to £7,521 p.a.

Further particulars may be obtained om Mrs. P. Vaughan-Smith, Staffing epartment, The University, outhampton, SO9 5NH, to whom plications including the names of two ferees, should be sent as soon as possible. lease quote ref: 1105/R/N. 3589(A)

ST THOMAS' HOSPITAL LONDON SE1

POSTDOCTORAL RESEARCH FELLOW

£5805 — £6252 inc.

To work for 3 years from April 1980 in the Vision Research Unit of the Rayne Institute, on neurochemical transmission in the mammalian retina.

Please write giving full curriculum vitae and names and addresses of two referees to Dr H. Ikeda, Head of Vision Research Unit, The Rayne Institute, St Thomas' Hospital, London SE1 7EH. 3539(A)

UNIVERSITY OF CAMBRIDGE

CAVENDISH LABORATORY

POST-DOCTORAL RESEARCH ASSISTANT

is required for research into the structure of metallic and oxide glasses. Experience in specimen preparation, neutron or X-ray scattering and electron microscopy would be an advantage.

Salary on a scale to £6,066 p.a. U.S.S. membership. Applications should be addressed to The Secretary, Cavendish Laboratory, Madingley Road, Cambridge CB3 0HE and reach him by 22 November. 3593(A)

UNIVERISTY OF GLASGOW

DEPARTMENT OF NEUROLOGY

POSTDOCTORAL RESEARCH ASSISTANT

required for a three year project on myasthenia gravis and the antigenic structure of the human acetylcholine receptor. Experience of monoclonal antibody or tissue culture techniques preferable.

Salary according to age and experience. Applications, giving two referees, to Dr T. Barkas, Department of Neurology, Institute of Neurological Sciences, Southern General Hospital, Glasgow G51 4TF. (041-445-2466 Ext. 8036) 3591(A)

UNIVERSITY OF GLASGOW

LECTURESHIP IN PHARMACOLOGY

Applications are invited from medically and/or scientifically qualified pharmacologists for a Lectureship in Pharmacology. Salary will be within range £4,333 to £6,355 of the Lecturers' scale £4,333 to £8,992, with placement according to qualifications and experience.

Further particulars may be obtained from the Secretary of the University Court, (Room 18), University of Glasgow, Glasgow, G12 8QQ, with whom applications (8 copies), giving the names and addresses of three referees, should be lodged on or before 23rd November, 1979.

In reply please quote Ref. No. 4263/2M. 3579(A)

AUSTRALIAN NATIONAL UNIVERSITY

Applications are invited from suitably qualified persons for appointment to the following positions:

RESEARCH SCHOOL OF PHYSICAL SCIENCES

DEPARTMENT OF ENGINEERING PHYSICS

RESEARCH FELLOWS IN LASER-PRODUCED PLASMAS (Two positions)

Applicants should have a background of research into laser-produced plasmas and would be expected to participate in the existing experimental programmes as well as developing ongoing research projects in this field. Research within the Laboratory is supported by two single-beam Neodymium laser systems with peak powers of several hundred gigawatts. The main research areas are the spectroscopy of laser-produced plasmas and laser-matter interactions including self-focussing, field-gradient force effects, adsorption and reflectance mechanisms, wavelength scaling effects, and ionisation processes. Extensive diagnostic systems, computing facilities and electronic and mechanical engineering workshops are available to support the research.
CLOSING DATE: *31 JANUARY 1980.*

RESEARCH FELLOW IN IMAGE PROCESSING

This position, within an established group in the Department, involves study of image enchancement and analysis techniques (for example, with respet to spectral, temporal and spatial characteristics of LANDSAT imagery) and investigations of new remote sensing applications enabled by improvements in these techniques and in the availability of data.
The appointee would have access to the Department's flexible PD! 11/45 based interactive image analysis system, to the Research School of Physical Sciences DEC system 10 and to the ANU Computer Services Centre UNIVAC 1100/42 computers.
CLOSING DATE: *31 JANUARY 1980.*

RESEARCH SCHOOL OF SOCIAL SCIENCES

'AGEING AND THE FAMILY' PROJECT

RESEARCH FELLOWS/SENIOR RESERCH FELLOWS (two posts)

The School wishes to appoint an applied sociologist and a policy analyst to particpate in a study of the social relations and living arrangements of the aged, with particular emphasis on implications for policy.
Preferably, applicants will have a background in either applied social science or policy analysis. Experience in gerontology, the sociology of the family, survey analysis, or assessment of social welfare or housing programs would be a distinct advantage.
One of the appointees will be expected to take responsibility for co-ordinating the project. This person will be responsible for providing academic leadership, administering the budget, co-ordinating the activities of other staff, and co-operating with related efforts by others in the School and in other universities and institutions.
Further information is available from Dr L.H. Day in the Research School of Social Sciences of the University.
CLOSING DATE: *23 NOVEMBER 1979.*

URBAN RESEARCH UNIT

FELLOW/RESEARCH FELLOW/SENIOR RESEARCH FELLOW

Applications are invited from social scientists and others in related disciplines interested in multi-disciplinary research on Australian cities. Applicants should have a postgraduate degree and, for a Fellowship or Senior Research Fellowship, considerable experience in research.
Preference will be given to applicants interested in research related to public policy.
Up to three appointments may be made.
CLOSING DATE: *16 NOVEMBER 1979.*

Salary-on appointment will be in accordance with qualifications and experience within the ranges: Fellow $A18,990 to $25,441; Senior Research Fellow $A22,755 to $27,143; Research Fellow $A16,291 to $21,265 p.a. Present exchange rate — $A1 : 51p : $US1.12.

TERM OF APPOINTMENT: Fellow — five years with possibility of reappointment to age 65. Research Fellow/Senior Research Fellow — three years with possible extension to five years.
Reasonable appointment expenses are paid. Superannuation benefits are available for applicants who are eligible to contribute. Assistance with finding accommodation is provided for an appointee from outside Canberra. The University reserves the right not to make an appointment or to make an appointment by invitation at any time.
Prospective applicants should first obtain further particulars from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. 3596(A)

POSTDOCTORAL POSITION starting January 1980 to study molecular biology of mammalian mitochondrial DNA. Experience in recombinant DNA cloning and DNA sequencing is desirable but not necessary. Send curriculum vitae, a brief summary of past research and names of references to either Dr P.J. Laipis or Dr W.W. Hauswirth, Department of Biochemistry and Molecular Biology, Box J-245, JHMHC, University of Florida, Gainesville, Florida 32610. *Equal Employment Opportunity/Affirmative Action Employer* W473(A)

RESEARCH ASSOCIATE — POSTDOCTORAL — to work on the organization and transcription of the genes for mammalian low molecular weight RNA's or on the function of these RNA species. Recent PhD with background in molecular biology or biochemistry preferred. Send C.V. and names of three references to: Dr George Eliceiri, Cell Biology Division, Department of Pathology, St. Louis University School of Medicine, St. Louis University Medical Center, 1402 S. Grand, St. Louis, MO 63104, U.S.A. An Equal Opportunity Employer M/F/H. W468(A)

DIRECTOR

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Ann Arbor, Michigan

The Pharmaceutical Research Division of Warner-Lambert is seeking an outstanding scientist to head a newly constituted group. The new Director will be responsible for a group of 25-30 scientists dealing with absorption, distribution, metabolism and excretion of new drugs.

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- Extensive personal experience with biopharmaceutic and drug metabolism studies in the pharmaceutical industry.
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- Management and leadership skills with abilities to motivate staff to a high level of scientific performance and to communicate well.

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W479(A)

Warner-Lambert

UNIVERSITY OF OXFORD

PLANT PHYSIOLOGIST

with research experience required to work with the Reader in Soil Science on an ARC project studying root temperature effects on nutrient uptake.

Salary in Grade 1A: £4,333 to £7,521.

Applications, mentioning two referees, to the Administrator, Department of Agricultural Science, Parks Road, Oxford OX1 3PF before 10 November 1979.

3595(A)

UNIVERSITY OF NEWCASTLE UPON TYNE

DEPARTMENT OF MECHANICAL ENGINEERING

RESEARCH ASSOCIATE

Applications are invited for the temporary post of Research Associate for research into new types of gyroscopic instruments. The work is financed by the Science Research Council and will investigate the application of mechanical and electrical characteristics of selected crystals to the precise measurement of angular velocity and displacement. Applicants should be graduates in a relevant branch of applied physics or engineering. Experience in crystal mechanics and electronics will be an advantage.

The post is tenable for up to three years and the initial salary will be in the Range 1A scale: £4,333 to £7,521 per annum, according to age, qualifications and experience. Membership of the appropriate University superannuation scheme will be required.

Further particulars may be obtained from Professor L. Maunder, Department of Mechanical Engineering, The University, Newcastle upon Tyne, NE1 7RU, with whom applications (3 copies), together with the names and addresses of 3 referees should be lodged not later than 16th November, 1979. Please quote reference N.

3558(A)

GUY'S HOSPITAL

MEDICAL SCHOOL

SECRETARY

required as soon as possible to work for Profesor and Unit of Gastroenterology.

Applicants must be competent shorthand-typists. Salary according to experience and qualifications.

Apply in writing, stating age and giving details of qualifications and experience and the names of two referees, to the Secretary, Guy's Hospital Medical School, London Bridge, SE1 9RT, quoting Ref. G.R.1.

3566(A)

UNIVERSITY OF DUNDEE

Applications are invited for a newly established

CHAIR OF ZOOLOGY

in the Department of Biological Sciences. There is at present one other chair in the Department, the Boyd-Baxter Chair in Biology which is currently held by Professor W D P Stewart, FRS.

Further particulars are available from The Secretary, The University, Dundee DD1 4HN, with whom applications (ten copies or, if posted overseas one copy) should be lodged by November 22nd, 1979. Please quote reference EST/86/79J.

3576(A)

RESEARCH ASSISTANT

Postgraduate Research Biochemist/Physiologist/Pharmacologist required to join a research team investigating various aspects of cardiac biochemistry and pharmacology. Experience in spectrophotometry, tissue metabolite analysis or isolated tissue perfusion would be advantageous. The research unit is located in the new Rayne Institute and appointments will be made through St Thomas's Hospital Medical School.

Applications together with a curriculum vitae should be sent to Dr D J Hearse, The Rayne Institute, St Thomas' Hospital, London SE1.

3550(A)

UNIVERSITY OF SOUTHAMPTON

RESEARCH ASSISTANT — GRADUATE

to join a team working on immunochemical aspects of leukaemia and lymphoma. Appointment funded by MRC, for five years in the first instance.

Salary in range £3,775 to £4,910 p.a.

Further particulars may be obtained from Mrs. P. Vaughan-Smith, Staffing Department, The University, Southampton, SO9 5NH, to whom applications including the names of two referees, should be sent as soon as possible. Please quote ref: 1106/R/N. 3590(A)

ANNOUNCEMENTS

EUROPEAN SOCIETY FOR RADIATION BIOLOGY

The 15th Meeting of the European Society for Radiation Biology will be held at the Medical Faculty of the Erasmus University Rotterdam, the Netherlands from Augus 25 to 28, 1980. Further information can be obtained from the secretary of the meeting Dr J.J. Broerse, Radiobiological Institute TNO, P.O. Box 5815, 151 Lange Kleiweg 2280 HV Risjwijk, the Netherlands.

W465(G)

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FELLOWSHIPS

The Weizmann Institute of Science is pleased to announce the availability of the

Charles H. Revson Career Development Chairs and Post-Doctoral Fellowships.

The Revson Chairs are intended for promising young scientists who are eligible for appointment as Senior Scientists at the Weizmann Institute.

The Revson Fellowships will be awarded to promising young scientists within three years after they have received their PhD degrees.

Both categories are intended either for scientists residing in Israel or those who are considering making their residence there. These appointments may commence any time during 1980. The appointments for Revson Career Development Chairs are for a three to four year period. Appointees found suitable at the end of this period, will be invited to continue as permanent members of the Weizmann Institute's scientific staff.

Appointments for Revson Post-Doctoral Fellowships are for periods of one to two years.

Candidates are requested to write for application forms to:

Charles H. Revson Foundation Programme
c/o Academic Secretary
Weizmann Institute of Science
Rehovot,
ISRAEL

W466(E)

KING'S COLLEGE HOSPITAL MEDICAL SCHOOL

(University of London)
Denmark Hill, London,
SE5 8RX.

COWBURN RESEARCH FELLOWSHIP/ SCHOLARSHIP

Applications are invited from recent graduates with good honours degrees, and postgraduate students holding PhD or quivalent for the above post. The uccessful applicant will join a research group in the Department of Medical Microbiology investigating immune esponses to virus infections. The work will nvolve a detailed study of the nature of the ntibody response to a localised virus nfection in normal and immuno-ompromised hosts and the role of these esponses in limiting virus growth.

The successful applicant will be esignated fellow or scholar, depending on qualifications and experience and the post s tenable for three years. Salary will be on he University scales for Research and nalagous staff with the starting salary in he range of £4,515 to £5,362 including London Weighting.

Further details of the post and the project are available from Professor J.R. Pattison (01-274-6222 Extn. 2480) and pplications should be sent to the Secretary of the Medical School by 10th November 979. 3582(E)

UNIVERSITY OF BIRMINGHAM

DEPARTMENT OF MICROBIOLOGY

RESEARCH FELLOWSHIP

Applications are invited for a three-year MRC-financed research fellowship. Salary will be on the RF1A scale £4333 to £7521, plus superannuation. Starting salary will be up to £5488 according to age and previous (not necessarily related) postdoctoral experience.

The project is to study the chemical changes in virus-infected cells both *in vitro* and *in vivo* by X-ray microanalysis. The work will be carried out in two laboratories under the supervision of Dr J Stephen (Department of Microbiology, Birmingham University) and Dr T.A. Appleton (Department of Physiology, Cambridge University). Full provision has been made for travel between the two institutions and stays of short duration in Cambridge.

Further particulars are available from the Assistant Registrar, Science and Engineering, University of Birmingham, PO Box 363, Birmingham B15 2TT, to whom applications (three copies), including full curriculum vitae and naming three referees should be sent by Friday 16th November, 1979. Please quote reference NM6(r). 3573(E)

UNIVERSITY OF DUNDEE

DEPARTMENT OF BIOCHEMISTRY

Applications are invited for a

TEMPORARY LECTURESHIP/ POSTDOCTORAL FELLOWSHIP

in Biochemistry available in the above Department for two years (Lectureship for nine months, followed by MRC Postdoctoral Fellowship for 15 months) from 1 January 1980 (or as soon as possible thereafter).

The successful candidate will be required to undertake teaching duties and to contribute to the research activities of a group studying the genetic deficiency and regulation of hepatic microsomal UDP-glucuronyltransferas and glucose-6-phosphatase.

Applicants should be scientists with an appropriate PhD qualification. Postgraduate and/or up to three years' postdoctoral experience in enzymology, protein chemistry, membrane proteins, and/or immunology would be useful.

Salary dependent on qualifications and experience, but an an appropriate point within the range £4333 to £5488.

Applications (with a curriculum vitae and the names of two referees) should be sent by 15 November 1979 to The Secretary, The University, Dundee DD1 4HN. Please quote Reference EST/91/79H. Professor P.B. Garland, Head of the Department of Biochemistry (telephone Dundee 23181 ext 595) will be pleased to answer informal enquiries.

3575(E)

GIRTON COLLEGE CAMBRIDGE

Applications are invited for a scientific research fellowship open to graduates of any university and tenable for three years from 1st October 1980. The applications should be in the field of Mathematics, Natural Sciences, Geography and allied subjects.

The fellowship is to the value of £3,000 per annum to a candidate with a PhD and £2,750 per annum to a candidate who has not completed a PhD. The fellowship is pensionable under U.S.S.

Particulars are available from the Secretary to the Council, Girton College, Cambridge CB3 0JG to whom applications should be sent by 19th November, 1979. 3503(E)

UNIVERSITY OF ESSEX

FELLOWSHIP

Laser-produced plasmas

An S.R.C. — sponsored post-doctoral Fellowship is available, initially for two years from 1 January 1980, to work under the supervision of Dr T.A. Hall on Rayleigh Taylor instabilities in laser-produced plasmas. The experiments require the development of a high-speed X-ray framing camera and previous experience with high speed electronic streak cameras would be an advantage. Starting salary up to £4,910 per annum on Range IA.

Applications (two copies), including a curriculum vitae and the names and addresses of two referees, should reach Mrs D. Weatherall, Department of Physics, University of Essex, Wivenhoe Park, Colchester CO4 3SQ, from whom further particulars may be obtained, by November 16, 1979. 3562(E)

Please mention

Nature

when replying to
these advertisements

STUDENTSHIPS

UNIVERSITY OF DURHAM

ENGINEERING GEOLOGY LABORATORIES

SRC CASE RESEARCH STUDENTSHIP

Applications invited from engineers or geologists, holding or expecting to obtain by the end of this year a good honours degree, for a project, starting 1 January 1980, on the effects of ground movements caused by excavation. The cooperating body is the Northumbrian Water Authority.

Further information and enquiries to Dr P B Attewell, Engineering Geology Laboratories, University of Durham, South Road, Durham DH1 3LE, (Tel. Durham (0385) 64971 Ext. 411), by 10 November, 1979. 3584(F)

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university college of swansea

Department of Physics

SRC RESEARCH STUDENTSHIPS

We have two vacancies for post-graduate research students. One in **Medical Physics** to join a group carrying out research in co-operation with the Medical Physics Department of the nearby Singleton Hospital on the *in vivo* detection of elements in the human body using neutron activation analysis; the other to join the **Ionization Physics** research group to work on processes of ionization and attachment in pulsed neutron counters in collaboration with UKAEA, Winfrith. SRC Case Studentships are available for both posts.

Applicants should possess a good Bachelor's or Master's degree from a British University in Physics, Electrical Engineering or related subject, and, if appointed, will be encouraged to register for a PhD degree of the University of Wales.

Further details from Professor J Dutton, Telephone (0792) 25678, Extn 390. 3580(F)

ASSISTANTSHIPS

UNIVERSITY OF DUBLIN
Trinity College

POST-DOCTORAL RESEARCH

Applications are invited for a post-doctoral research assistantship to work on the structure and function of tryptophan synthetase in yeast with Dr C Bailey. The enzyme has been purified and shown to be a multi-functional protein. The research will be into the structural integrity, sterochemical relationship and interconnection of the two active sites present and applicants should preferably have experience in protein chemistry and/or enzymology. The project is supported by the National Board for Science and Technology for a period of three years, on a salary scale £4,182 to £4,879.

Candidates should submit a curriculum vitae, together with the names and addresses of two referees to:

The Establishment Officer, Staff Office, West Theatre, Trinity College, Dublin 2. 3545(P)

LEICESTER POLYTECHNIC

SCHOOL OF CHEMISTRY

LEA RESEARCH ASSISTANTSHIP

This is a three year post and the appointee will be registered for a CNAA PhD degree under the supervision of Dr Roger Linford, Reader in Chemistry. Duties will include six hours per week teaching. The project is concerned with fundamental aspects of the behaviour of solid electrolytes and related battery systems intended for heart pacemaker and similar applications. Previous experience in solid state electrochemistry not necessary, but an interest in structural inorganic Chemistry, X-ray powder diffraction or differential thermal analysis an advantage. A good honours degree or equivalent in Chemistry, materials Science or a related subject required.

Commencing salary: £2,913 plus £72 supplement p.a.

Application forms and further details from Staffing Officer, Leicester Polytechnic, PO Box 143, Leicester LE1 9BH. Informal enquiries to Roger Linford, Tel: (0533) 551551 Ext. 2202. 3574(P)

ASSOCIATESHIPS

UNIVERSITY OF NOTTINGHAM

DEPARTMENT OF ZOOLOGY

POST-DOCTORAL RESEARCH ASSOCIATE IN MEMBRANE BIOPHYSICS/PHARMACOLOGY

Applications are invited for an SRC-supported post-doctoral associateship commencing 1st January, 1980, or as soon as possible thereafter, to work with Professor PNR Usherwood on single ionic channels in locust muscle membrane gated by amino acid receptors. Ideally the successful applicant will have experience in membrane biophysics or pharmacology and an interest in computing.

The appointment is tenable for one year initially with a possibility of extension for a further year. The starting salary will be between £4,910 to £6,066.

Further details of this vacancy may be obtained from Professor Usherwood.

Applications, including curriculum vitae and the names and addresses of two referees, should be sent to: The Departmental Secretary, Zoology Department, Nottingham University, University Park, Nottingham NG7 2RD. 3559(O)

AWARDS

Research into Renal Diseases in the Tropics

Competitive Award of up to £150,000

The Wellcome Trustees wish to encourage research into diseases occurring in the Tropics which affect the renal system. A competitive award of up to £150,000 is offered for a period of not more than 5 years.

Preference will be given to proposals which demonstrate an interdisciplinary approach and especially those which include sustained collaboration with research centres in the Tropics. This award is additional to the Trustees' normal arrangements for supporting tropical medical research under which smaller grant applications can be considered.

Applications must be submitted on the appropriate form. Please telephone or write (quoting Ref: RDT/N) to:

The Grants Section,
The Wellcome Trust, 1 Park Square West,
London NW1 4LJ. Tel: 01-486 4902.

The closing date for applications is 11th Jan. 1980.

The Wellcome Trust

3556(N)

WYE COLLEGE
(University of London)

Applications are invited for an SRC CASE award held jointly between Rank Hovis McDougall and the Department of Plant Sciences at Wye College.

The award is for work on the physiology of alternative growth habitats in field bean and concerns recent genetic developments for the adaptation of this traditional crop to modern intensive agriculture.

Applicants should have at least an upper second class degree in Agriculture, or Plant Sciences or a related area.

Applications should be made to Dr G.P. Chapman, Department of Plant Sciences, Wye College, University of London, Ashford, Kent TN25 5AH. 3554(N)

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Applications to attend and requests for further information should be sent to Professor R B Cundall, Department of Biochemistry, University of Salford, Salford M5 4WT, UK, applications to arrive before December 15, 1979. Up to 90 places are available for post-graduate and both junior and senior post-doctoral applicants who should append *where appropriate* a curriculum vitae (including references to recent publications in these or allied areas) and, for post-graduates, a letter of recommendation from their supervisor and/or Head of Department. Successful applicants from NATO countries may receive some assistance towards accommodation and travel costs. Depending on response, a small number of places for applicants from non-NATO countries may also be available. 3586(D)

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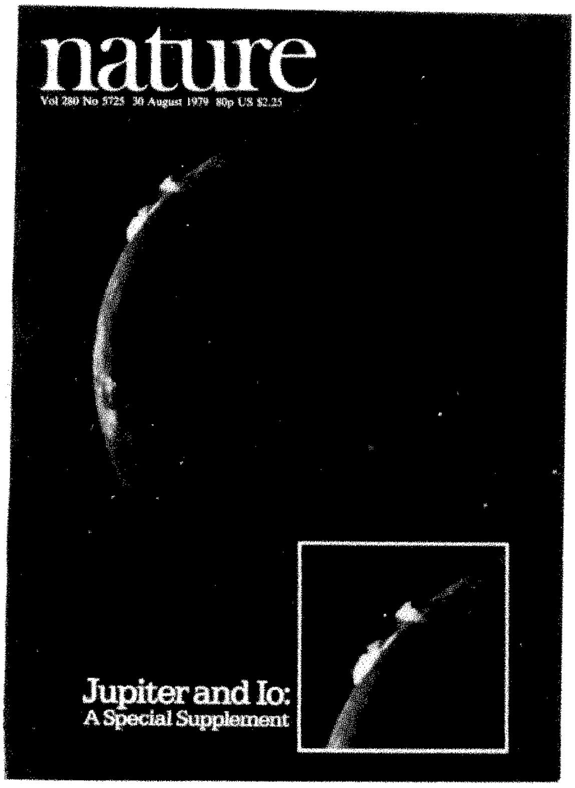

VOYAGER I

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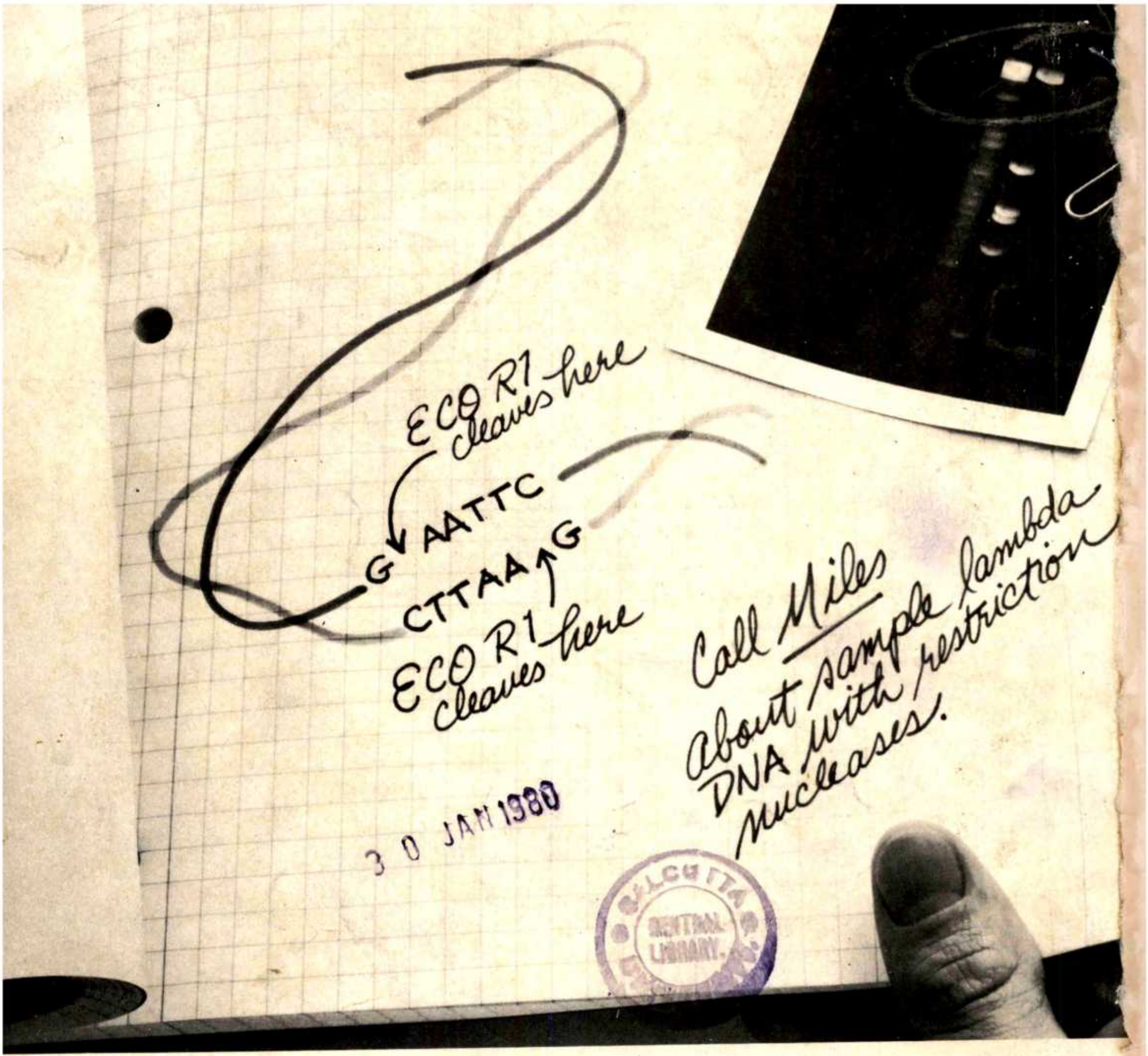

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Miles Laboratories, Inc.
P.O. Box 2000
Elkhart, IN 46515
Tel: 219-264-8804

Miles Laboratories, Ltd.
P.O. Box 37
Stoke Court, Stoke Poges
Slough SL2 4LY, England
Tel: Farnham Common 2151

Research Products Division MILES

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